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(54) Title: GENERATION OF SURFACE COATING DIVERSITY

(57) Abstract: The present invention relates to a surface discovery system comprising chemical compositions and high-throughput combinatorial synthesis methods for generating large numbers of diverse surface coatings on solid substrates. This surface discovery platform is built upon a fundamental chemical unit referred to as a synthon. Each synthon comprises at least three elements: a chemical backbone coating on the solid substrate that comprises a passive (P) constituent and an active (A) constituent; a spacer unit (S) separating the backbone from a functional group; and a functional group (F). Variation of these synthon elements allows generation of large libraries surface coatings with a broad range of molecular and macroscopic properties. Further the spectrum of surfaces provided by the invention permits optimization of the wide range of solid-phase applications that involve surface immobilization of molecules.

GENERATION OF SURFACE COATING DIVERSITY

FIELD OF THE INVENTION

The present invention relates to surface coating technology. In particular, the invention relates to a method for generating a library of different surface coatings on a substrate, to a method for optimising a substrate surface for a solid phase application and arrays or beads possessing discrete regions of particular optimised surface coatings.

BACKGROUND OF THE INVENTION

Current surface coating technology provides a relatively limited number of established surfaces that may be used in new solid-phase chemical or biochemical applications. The lack of established surfaces stems primarily from the difficulty associated with the generation of different surface coatings. While large numbers of chemically diverse compounds may now be generated in solution without too much difficulty, the ability to graft these molecules on to a solid phase and create a large number of surface coatings has proven a much more difficult problem to solve. In particular, the chemistry of grafting molecules onto solid phases to create surface coatings is highly unpredictable, and has to date remained more an art than a science.

There are numerous applications where a diverse range of novel surface coatings would be particularly advantageous, for example in the area of solid phase biological assays. With the number of novel proteins growing each day, there is growing need for novel solid phase surfaces that are compatible with the immobilization of these complex macromolecules. Despite this need, in practice there are to date relatively few solid surfaces available across the wide range of solid phase applications used to study biological molecules. For example, in the area of capture and display of biomolecules each commercial supplier has its own particular solid phase surface embodiment that is prescribed across a broad range of specific applications. One specific example is a surface generated using the well-established PEG chemistry as described in an article by Ruiz-Taylor *et al.* ("Monolayers of derivatized poly(L-lysine)-grafted poly(ethylene glycol) on metal oxides as a class of biomolecular interfaces," PNAS USA 98: 852–857 (2001)).

Another example is the relatively new boronic acid complex chemistry used to prepare surfaces for immobilization of proteins described by Stolowitz *et al.* ("Phenylboronic Acid-Salicylhydroxamic Acid Bioconjugates. 1. A Novel Boronic Acid Complex for Protein Immobilization," Bioconjugate Chemistry 12: 229-239 (2001)).

Surface plasmon resonance (SPR) has now been widely adopted as a technique for detecting protein-ligand and protein-protein binding interactions. However the utility of SPR with a particular protein system depends greatly on the vagaries of how that macromolecule binds to the surface of the solid substrate when immobilized. If a particular SPR surface causes a protein of interest to bind in an orientation that is unfavorable for detecting ligand binding, there are only a handful of alternative surfaces with a limited range of binding properties from which to choose (see, e.g. Rich and Myszka "Advances in surface plasmon resonance biosensor analysis," Current Opinion in Biotechnology 11: 54–61 (2000)).

Similarly, mass spectrometry also is now widely employed for the analysis of biological macromolecules. These methods typically involve immobilization of a protein on a surface of substrate where it is then exposed to a ligand binding interaction. Following ligand binding (or non-binding) the molecule is desorbed from the surface and into a spectrometer using a laser (see, e.g. Merchant and Weinberger, "Recent advancements in surface-enhanced laser desorption/ionization-time of flight-mass spectrometry," Electrophoresis 21: 1164–1177 (2000)). As in the SPR experiment, the success of the mass spectrometry experiment depends largely on the interaction between the immobilized protein and the surface. In view of the thousands of proteins with different surface interactions, there is clearly a need for a large number of different substrate surfaces in order for mass spectrometry to be applied successfully to the high throughput analysis of the proteome.

Accordingly, the inability to provide a diverse array of surface coatings stands as an impediment to development in solid phase biological technologies such as biological assays and diagnostics, and biomaterials. Such an impediment also extends across a broad spectrum of other technologies, ranging from solid-phase chemical synthesis, catalysis development and separation and purification technologies.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a method of generating a library of different surface coatings on a substrate comprising:

a) selecting a surface coating synthon of formula B-S-F, wherein B is a copolymer of at least one passive constituent P and at least one active constituent A, S is a spacer unit and F is a chemical or biological functional group, wherein S is attached to an active constituent A of copolymer B, and wherein the synthon has at least one point of diversity selected from P, A, S and F;

b) applying backbone coating(s) of the selected copolymer B onto a substrate;

c) attaching the selected combination(s) of spacer unit S and functional group F to an active constituent A of copolymer B according to said selected synthon;

wherein steps b) and c) are performed such that surface coatings according to the synthon are generated on localised regions of the substrate, thereby providing said library of different surface coatings on the substrate.

In another aspect, the present invention provides a method of optimizing a substrate surface for a solid-phase application involving immobilization of a molecule comprising:

a) generating a library of different surface coatings on a substrate by a method comprising:

1) selecting a surface coating synthon of formula B-S-F, wherein B is a copolymer of at least one passive constituent P and at least one active constituent A, S is a spacer unit and F is a chemical or biological functional group, wherein S is attached to an active constituent A of copolymer B, and wherein the synthon has at least one point of diversity selected from P, A, S and F;

2) applying backbone coating(s) of the selected copolymer B onto a substrate;

3) attaching the selected combination(s) of spacer unit S and functional group F to an active constituent A of copolymer B according to said

selected synthon;

wherein steps 2) and 3) are performed such that surface coatings according to the synthon are generated on localised regions of the substrate, thereby providing said library of different surface coatings on the substrate;

b) exposing at least two of the surface coatings in the library to the molecule to be immobilized; and

c) determining which of the at least two surfaces results in better performance of the immobilized molecule in the solid-phase application.

In a further aspect, the present invention provides a biological molecule detection unit capable of detecting at least two biological molecules, said unit comprising a substrate having a plurality of surface coatings wherein at least two of said coatings are different, and tailored to recognise, bind to or associate with a particular biological molecule. A person skilled in the art would be able to adapt the methods described herein to prepare such a detection unit.

The present invention provides a method for generating a library of different surface coatings on a substrate which can be advantageously used as part of a surface discovery system. The library is generated using a unique synthon approach that provides an architectural framework from which the specific surface coatings can be realised.

The present invention fills a critical gap in solid surface technology by providing a high-throughput platform for the rational generation and exploration of surface coatings with novel molecular and macroscopic properties. The diverse combinatorial libraries of surface coatings that may be generated in a high-throughput manner using the synthon-based approach disclosed herein may be applied across a broad spectrum of technologies, ranging from solid-phase chemical synthesis, catalysis development, separation and purification technologies, biological assays and diagnostics, and biomaterials development.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The Synthon

As used herein the term "synthon" is used to refer to a fundamental chemical unit, or building block, which provides an architectural framework to design and develop a diverse array of surface coatings on a substrate. The synthon comprises three basic

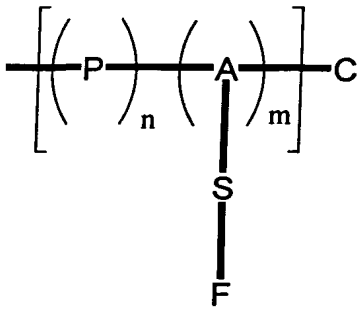
elements and can simplistically be represented as B-S-F, wherein B is a copolymer of at least one passive constituent P and at least one active constituent A, S is a spacer unit and F is a chemical or biological functional group. The spacer unit S is attached to an active constituent A of copolymer B, and the synthon has at least one point of diversity selected from P, A, S and F.

Together, the space unit and the functional group form a "functional tether" that may be modified further with chemical entities. Simple combinatorial chemical variation of the four points of diversity (i.e. passive constituent, active constituent, spacer unit, and functional group) of the synthon described above allows one to generate potentially thousands of unique but related surfaces. Systematic variation of the active constituent, passive constituent, spacer unit and functional group allows generation of libraries of different surface coatings that span a spectrum of microscopic and macroscopic properties. These libraries of surfaces may be further explored using a variety of analysis techniques to discover the optimal surface for a variety of applications. Consequently, the synthon-based approach to generating surface coating diversity described herein provides a platform akin to combinatorial synthesis of small molecules and peptide libraries.

Although combinatorial approaches to generating molecular diversity have been employed to generate new lead compounds in the drug discovery process, these strategies have not to date been employed in the search for novel surface coatings that exhibit advantageous properties. Indeed, the standard solid phase combinatorial chemistry approaches used in drug discovery focus on generating variety in the small molecule properties and avoid diversity in the solid phase to which it is attached. The solid phase is viewed simply as a convenient handle to be disposed of after cleavage of the small molecule. Consequently, there has been little systematic exploration of solid phase surfaces and how their properties may be varied to optimize solid phase applications.

Together, the space unit and the functional group form a "functional tether" that may be modified further with chemical entities. Scheme 1 below illustrates a more detailed representation of a potential structure of the synthon.

Scheme 1



In scheme 1, the synthon further comprises a control agent C which may be optionally attached to copolymer B, as represented by -[P-A]-. The control agent C may be used as a means to prepare copolymer B under living/controlled polymerization conditions, or alternatively as a means to modify copolymer B. Preferred control agents include, but are not limited to, RAFT control agents, ATRP control agents, and nitroxide control agents. The use of a control agent advantageously provides a means to carefully control and design the molecular architecture of copolymer B, for example by controlling molecular weight distribution and/or distribution of monomeric units within the copolymer chain.

Simple combinatorial variation of the four points of diversity (i.e. passive constituent, active constituent, spacer unit, and functional group) that form the basic synthon described above allows one to generate potentially thousands of unique but related surfaces. In one preferred embodiment, the diversity is derived solely from the spacer unit S. In another preferred embodiment, the diversity is derived solely from the functional group F. In yet another preferred embodiment, the diversity is derived from both the spacer unit S and the functional group F.

In a relatively simple example, starting with one backbone coating on a base material (i.e. where the P and A constituents are kept constant) treatment with at least ten spacer unit S variants, and 10 different transformations of the functional group F, results in 100 different surfaces.

Of course, greater numbers of diverse compounds may be achieved if a control agent C is incorporated as another point of diversity. The control agent may be used as the start site for living-controlled polymerization reactions. Consequently, the backbone

coating may be modified by living-controlled polymerization independent of modifications at the spacer attached to the active constituent of the backbone.

Additionally, diversity may be achieved by utilizing orthogonal reaction strategies and/or combining mixtures of elements in building the synthons. For example, the passive constituent may act as a second active constituent by modifying it using a reaction orthogonal to that used to modify the first active constituent. Consequently, in some embodiments both the active and passive constituents may be modified with spacers to generate greater surface diversity.

Advantageously, the present invention allows construction of libraries comprising preferably at least 10, more preferably at least 100, still more preferably at least 1000, most preferably at least 10,000 different surface coatings.

Preferably, the library in accordance with the present invention is prepared in a multiplex format, and the library is also used in a multiplex format.

The Backbone Coating and its Parameters

The present invention involves applying backbone coating(s) of the selected copolymer B onto a substrate. The backbone coating provides the macroscopic design element in the method and is preferably covalently bound to the underlying substrate. In a preferred embodiment, the backbone coating is bound to the underlying substrate through well-known methods of polymer grafting, or other methods of coating a solid substrate such as dip coating, plasma polymerization, vapor deposition, stamp printing, gamma irradiation, electron beam exposure, thermal and photochemical radiation.

As the backbone coating, copolymer B comprises at least one passive constituent P and at least one active constituent A. These constituents may be viewed as monomeric units within the copolymer B. The copolymer B may also comprise other monomeric units. In some embodiments, the backbone coating may comprise more than one active and more than one passive constituent. As described in greater detail below, the active and passive constituents may be selected from a wide spectrum of compounds well-known in the art. Preferred are those compounds amenable to grafting or other methods of coating a solid substrate (e.g. dip coating, plasma polymerization, vapor deposition, stamp printing, gamma irradiation, electron beam exposure, thermal and photochemical radiation).

Generally, the backbone coating may be attached to the underlying substrate through either the active or passive constituent. In some embodiments, both constituents may engage in bonding interactions with the substrate.

The Active Constituent

The role of the active constituent is to provide a point for future diversity and would be represented by a functional group that is well known in the art to under go a vast number of chemical transformations, such as an amine, hydroxyl, anhydride, ester, carboxylic acid, ketone, epoxide, isocyanate and so on. Many well-known chemical monomers may be employed as active constituents in the formation of a synthon backbone coating. Selection of a particular set of active constituents may depend on the passive constituents selected and the desired chemistry for applying the backbone coating to the substrate.

Generally, the active constituent comprises a chemical moiety, or substituent group that may be chemically modified with a spacer compound (see described below).

For example, in an embodiment where gamma-initiated free-radical grafting is employed, one could employ any of the following monomers as the active constituent in the backbone coating: hydroxyethyl methacrylate, maleic anhydride, N-hydroxysuccinimide methacrylate ester, methacrylic acid, diacetone acrylamide, glycidyl methacrylate, PEG methacrylate.

In an alternative embodiment, more than one different active constituent may be present in the same backbone coating. For example, the coating may be made using a mixture of two active monomers. Once prepared, using well-known orthogonal approaches to chemical transformations, it is possible to differentially modify each of the different active constituents in the presence of the others, in a sequential and predetermined manner.

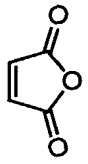
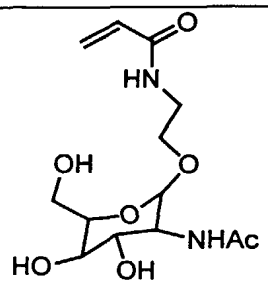
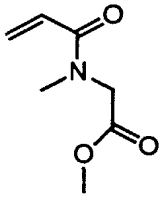
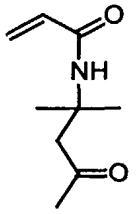
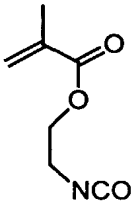
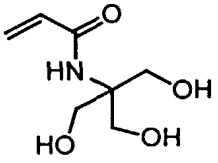
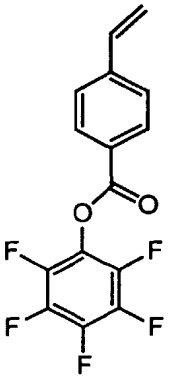
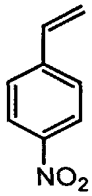
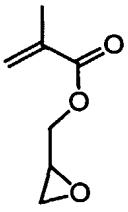
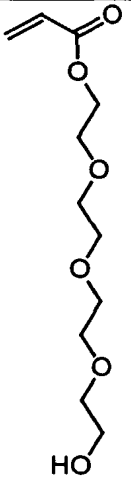
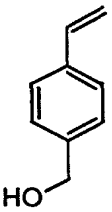
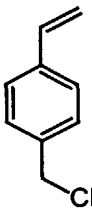
In preferred embodiments the active constituent comprises a chemical moiety, or substituent group that is amenable to surface grafting methods known in the art.

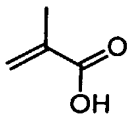
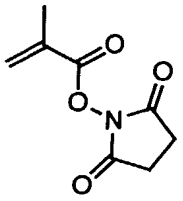
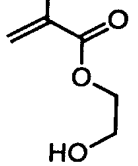
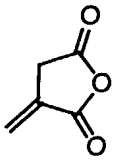
Table 1 below lists an exemplary selection of chemical monomers that may be used to provide the active constituents in the present invention. The compounds in this table are not intended to be limiting. Many common chemical variants of these compounds, as well

as, other compounds not listed here but well-known in the art of surface modification may also be used.

Preferably, copolymer B comprises an active constituent A derived from the polymerised residue of maleic anhydride.

Table 1: Selection of Active Constituents

ACTIVE	1	2	3	4
A				
B				
C				

D				

The Passive Constituent

Whereas the active constituent acts primarily as the point of attachment of the spacer, the primary role of the passive constituent is modification of molecular or macroscopic environment of the surface coating. For example, a set of passive constituents may be selected that modify the charge or the hydrophilicity of the surface coating. Modifications to passive constituents in a three dimensional stable network forming a surface coating allows determination of optimal surface properties for solid-phase applications. For example determination of a surface that allows binding of non-contiguous epitopes of a biomolecule so that they are available for a binding assay.

Further, the passive constituent also may act as a spacer unit for the active composition of the coating, in order to distribute the active group alternating, randomly, statistically or in a gradient fashion throughout the coating.

As mentioned above, in some embodiments the passive constituent may serve double-duty, also acting as a second active constituent for attachment of a spacer unit. Consequently, in some embodiments the passive constituent comprises a chemical moiety, or substituent group that may be chemically modified with a spacer compound. In this embodiment the reaction conditions for modification of the passive constituent are preferably orthogonal to those used to attach a spacer to the first active constituent.

The chemistry of the passive constituent may be provided by well known chemical monomers (preferably those that are commercially available) such as: styrene, dimethyl acrylamide, acrylonitrile, N,N dimethyl (or diethyl) ethyl methacrylate, 2-methacryloyloxy-ethyl-dimethyl-3-sulfopropyl-ammounium hydroxide, and methoxy PEG methacrylate. Preferably, copolymer B comprises a passive constituent B derived from the polymerised residue of styrene.

In preferred embodiments the passive constituent comprises a chemical moiety, or substituent group that is amenable to surface grafting methods known in the art.

Table 2 below lists a selection of chemical monomers that may be used to provide the passive constituents of the present invention. The compounds in this table are not intended to be limiting. Many common chemical variants of these compounds, as well as, other compounds not listed here but well-known in the art of surface modification may also be used.

Table 2: Selection of Passive Constituents

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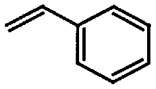
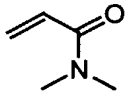
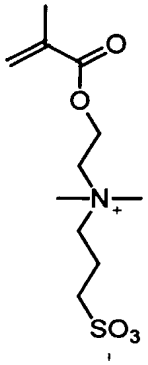
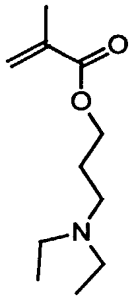
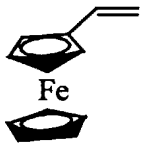
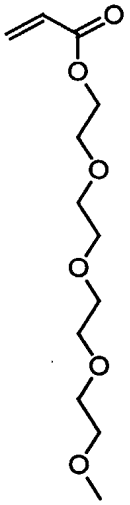
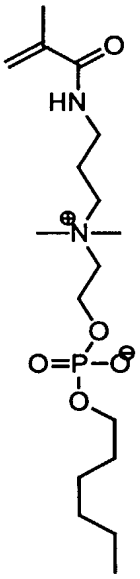
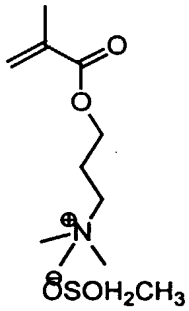
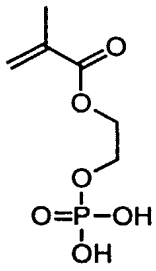
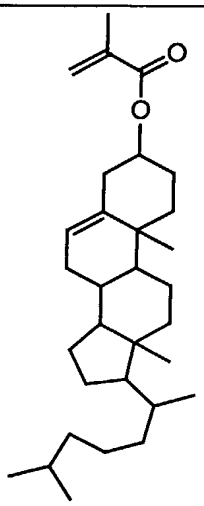
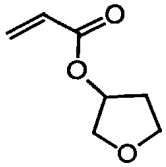
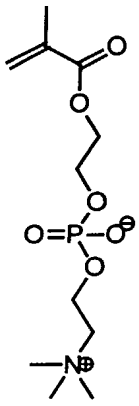
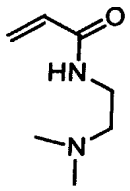
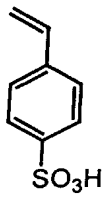
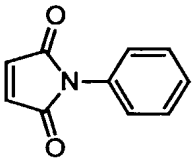
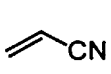
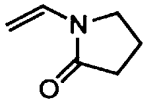
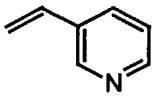
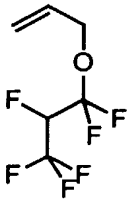
A					
B					

C					
D					

In an alternative embodiment, the desired macroscopic property of a surface coating for a selected solid phase application may be derived by *in silico* analysis of a range of synthon structures. Based on the *in silico* results, a passive constituent monomer with the chemical features necessary to generate the macroscopic property may be synthesized. Alternatively, the appropriate chemical features of the passive constituent may also be derived by *in situ* chemical transformation of an already applied backbone coating. In preferred embodiments, such *in situ* transformations of the passive backbone constituent are carried out in an orthogonal reaction scheme in order to maintain the integrity of the active constituent.

Application of the Backbone Coating

Generally, the synthon backbone coating may be applied to the substrate using any of the vast assortment of surface modifications methods present in the art (e.g. dip coating, plasma polymerization, vapor deposition, stamp printing, gamma irradiation, electron beam exposure, thermal and photochemical radiation).

In one embodiment, the backbone coating is polymerized from the constituent

monomers on the solid substrate using chemistry well-known in the art. A wide range of polymerization processes present in the art may be utilized. For example, controlled and/or living polymerization techniques of cationic, anionic, radical (such as NMP, ATP, ATRP, RAFT, Iniferter), condensation, and metathesis (such as ROMP and ADMET) all may be used. Non-controlled methods of polymerization well known in the art may also be utilized with this invention.

In one preferred embodiment, the backbone coating may be provided by methods known to afford living polymerization. By definition, the end groups of such living polymers have the ability to be further transformed, either by addition of a monomer to extend the macromolecule with the same monomer, a mixture of monomers or new monomeric compositions. Also, the end groups may be modified using any of a variety of organic chemistry transformations well-known in the art of small molecule manipulation.

In embodiments where the synthon includes a control agent (C) end group on the backbone, living-controlled polymerization may be used to further modify the backbone coating. Control agents and methods of conducting living-controlled polymerization are well-known in the art. Methods of living-controlled polymerization and re-initiation on the surfaces of non-functionalized solid substrates is described in co-pending U.S. patent application 10/109,777 filed March 28, 2002. Also, see, e.g. Canadian Patent applications 2,341,387 and 2,249,955 which disclose methods of living-controlled polymerization on solid polymer substrates.

Alternatively, the backbone coating may be applied to the substrate as a polymer solution, comprising macromers that will allow tethering by complementary chemistry to the surface of the substrate or encourage entanglement of the polymer in solution with the substrate. In the case of a macromer solution, the reactive units of the macromer may either be present at the end groups, or spaced throughout the backbone of the macromer in a random, block, or gradient fashion.

Preferably, the backbone coating is polymerised from constituent monomers to provide an alternating or block copolymer. The alternating, or substantially alternating character, of the copolymer is believed to provide an important spatial arrangement of the passive and active constituents which facilitates good surface coating of the substrate. Those skilled in the art will understand the degree of regularity necessary in order for a

copolymer to be considered of alternating character. It is preferred that the alternating copolymer has an alternating character defined by greater than 70 % of consecutive comonomer residue units being alternate between residues of the first comonomer and the second comonomer, more preferably greater than 90%. The block nature of the copolymer may also vary in an alternating fashion.

Preferably, the backbone coating is is a copolymer of maleic anhydride and styrene.

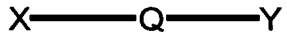
The Spacer

The spacer group provides a synthetic "handle" by which functional groups may be attached to the active constituent of the backbone coating.

As used herein, the term "spacer," "spacer molecule" and "spacer unit" are used interchangeably. As used herein, the term "functional tether" is used to refer to the combined moiety of a spacer molecule modified with the desired functional group for the synthon.

In one preferred embodiment, the spacer molecule may be represented by the generic structure shown in Scheme 2:

Scheme 2



Generally, both X and Y comprise chemical moieties or substituent groups that may be chemically modified independently, sequentially or under orthogonal conditions. For example, X may chemically react with the active constituent A to attach the spacer to the backbone. Subsequently, Y may be chemically modified with a desired functional group F.

Typical species may include for example, spacer molecules wherein X is the residue of an amino, hydroxyl, thiol, carboxylic acid, anhydrides, isocyanate, sulfonyl chloride, sulfonic anhydride, chloroformate, ketone, or aldehyde; Y is the same as defined for X; and Q is a linear or branched divalent organic group; and X and Y are not reactive with each other or Q. Preferably Q is selected from optionally substituted C_1 to C_{20} alkylene, optionally substituted C_2 to C_{20} alkenylene, optionally substituted C_3 to C_{20} cycloalkylene, optionally substituted C_2 to C_{20} alkynylene and optionally substituted C_6 to

C_{20} arylene, wherein one or more carbon atoms may be substituted with a heteroatom selected from O, S or N.

By "optionally substituted" is meant that a group may or may not be further substituted with one or more groups selected from, but not limited to, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, acetyleno, carboximidyl, haloaryloxy, isocyano, cyano, formyl, carboxyl, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, imino, alkylimine, alkenylimine, alkynylimino, arylimino, benzylimino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulphonyl, arylsulphonyl, alkylsolphinyl, arylsulphinyl, carboalkoxy, alkylthio, benzylthio, acylthio, sulphonamido, sulfanyl, sulfo and phosphorus-containing groups, alkoxysilyl, silyl, alkylsilyl, alkylalkoxysilyl, phenoxysilyl, alkylphenoxysilyl, alkoxyphenoxysilyl, arylphenoxysilyl, allophanyl, guanidino, hydantoyl, ureido, and ureylene. A carbon atom is considered to be substituted if it has a double bond to a heteroatom, such as oxygen, sulfur or nitrogen to form a carbonyl, thiocarbonyl or imine group, respectively.

In the above definitions the terms "aryl" and "heteroaryl" refer to any substituent which includes or consists of one or more aromatic or heteroaromatic ring respectively, and which is attached via a ring atom. The rings may be mono or polycyclic ring systems, although mono or bicyclic 5 or 6 membered rings are preferred. Examples of suitable rings include but are not limited to benzene, biphenyl, terphenyl, quaterphenyl, naphthalene, tetrahydronaphthalene, l-benzylnaphthalene, anthracene, dihydroanthracene, benzanthracene, dibenzanthracene, phenanthracene, perylene, pyridine, 4-phenylpyridine, 3-phenylpyridine, thiophene, benzothiophene, naphthothiophene, thianthrene, furan, benzofuran, pyrene, isobenzofuran, chromene, xanthene, phenoxathiin, pyrrole, imidazole, pyrazole, pyrazine, pyrimidine, pyridazine, indole, indolizine, isoindole, purine, quinoline, isoquinoline, phthalazine, quinoxaline, quinazoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, phenazine, isothiazole, isooxazole, phenoxazine and the like, each of which may be optionally substituted.

In the above definitions, the term "alkyl", used either alone or in compound words such as "alkenyloxyalkyl", "alkylthio", "alkylamino" and "dialkylamino" denotes straight chain, branched or cyclic alkyl, preferably C_{1-10} alkyl or cycloalkyl. Examples of straight chain and branched alkyl include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, amyl, isoamyl, sec-amyl, 1,2-dimethylpropyl, 1,1-dimethyl-propyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 1,1-dimethylbutyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2,-trimethylpropyl, 1,1,2-trimethylpropyl, heptyl, 5-methoxyhexyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 1,4-dimethyl-pentyl, 1,2,3,-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, octyl, 6-methylheptyl, 1-methylheptyl, 1,1,3,3-tetramethylbutyl, nonyl, 1-, 2-, 3-, 4-, 5-, 6- or 7-methyl-octyl, 1-, 2-, 3-, 4- or 5-ethylheptyl, 1-, 2- or 3-propylhexyl, decyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- and 8-methylnonyl, 1-, 2-, 3-, 4-, 5- or 6-ethyloctyl, 1-, 2-,3- or 4-propylheptyl, undecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8- or 9-methyldecyl, 1-, 2-, 3-, 4-, 5-, 6- or 7-ethylnonyl, 1-, 2-, 3-, 4- or 5-propyloctyl, 1-, 2- or 3-butylheptyl, 1-pentylhexyl, dodecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9- or 10-methylundecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- or 8-ethyldecyl, 1-, 2-, 3-, 4-, 5- or 6-propylnonyl, 1-, 2-, 3- or 4-butyloctyl, 1-2-pentylheptyl and the like. Examples of cyclic alkyl include mono- or polycyclic alkyl groups such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl and the like.

In the above definitions the term "alkoxy" denotes straight chain or branched alkoxy, preferably C_{1-10} alkoxy. Examples of alkoxy include methoxy, ethoxy, n-propoxy, isopropoxy and the different butoxy isomers.

The term "alkenyl" denotes groups formed from straight chain, branched or cyclic alkenes including ethylenically mono-, di- or poly-unsaturated alkyl or cycloalkyl groups as previously defined, preferably C_{2-10} alkenyl. Examples of alkenyl include vinyl, allyl, 1-methylvinyl, butenyl, iso-butenyl, 3-methyl-2-butenyl, 1-pentenyl, cyclopentenyl, 1-methyl-cyclopentenyl, 1-hexenyl, 3-hexenyl, cyclohexenyl, 1-heptenyl, 3-heptenyl, 1-octenyl, cyclooctenyl, 1-nonenyl, 2-nonenyl, 3-nonenyl, 1-decenyl, 3-decenyl, 1,3-butadienyl, 1-4,pentadienyl, 1,3-cyclopentadienyl, 1,3-hexadienyl, 1,4-hexadienyl, 1,3-

cyclohexadienyl, 1,4-cyclohexadienyl, 1,3-cycloheptadienyl, 1,3,5-cycloheptatrienyl and 1,3,5,7-cyclooctatetraenyl.

The term "alkynyl" denotes groups formed from straight chain, branched or cyclic alkyne including those structurally similar to the alkyl and cycloalkyl groups as previously defined, preferably C_{2-10} alkynyl. Examples of alkynyl include ethynyl, 2-propynyl and 2- or 3-butynyl.

The term "acyl" either alone or in compound words such as "acyloxy", "acylthio", "acylamino" or "diacylamino" denotes carbamoyl, aliphatic acyl group and acyl group containing an aromatic ring, which is referred to as aromatic acyl or a heterocyclic ring which is referred to as heterocyclic acyl, preferably C_{1-10} acyl. Examples of acyl include carbamoyl; straight chain or branched alkanoyl such as formyl, acetyl, propanoyl, butanoyl, 2-methylpropanoyl, pentanoyl, 2,2-dimethylpropanoyl, hexanoyl, heptanoyl, octanoyl, nonanoyl, decanoyl, undecanoyl, dodecanoyl, tridecanoyl, tetradecanoyl, pentadecanoyl, hexadecanoyl, heptadecanoyl, octadecanoyl, nonadecanoyl and icosanoyl; alkoxycarbonyl such as methoxycarbonyl, ethoxycarbonyl, t-butoxycarbonyl, t-pentyloxycarbonyl and heptyloxycarbonyl; cycloalkylcarbonyl such as cyclopropylcarbonyl, cyclobutylcarbonyl, cyclopentylcarbonyl and cyclohexylcarbonyl; alkylsulfonyl such as methylsulfonyl and ethylsulfonyl; alkoxysulfonyl such as methoxysulfonyl and ethoxysulfonyl; aroyl such as benzoyl, toluoyl and naphthoyl; aralkanoyl such as phenylalkanoyl (e.g. phenylacetyl, phenylpropanoyl, phenylbutanoyl, phenylisobutylyl, phenylpentanoyl and phenylhexanoyl) and naphthylalkanoyl (e.g. naphthylacetyl, naphthylpropanoyl and naphthylbutanoyl; aralkenoyl such as phenylalkenoyl (e.g. phenylpropenoyl, phenylbutenoyl, phenylmethacryloyl, phenylpentenoyl and phenylhexenoyl and naphthylalkenoyl (e.g. naphthylpropenoyl, naphthylbutenoyl and naphthylpentenoyl); aralkoxycarbonyl such as phenylalkoxycarbonyl (e.g. benzyloxycarbonyl); aryloxycarbonyl such as phenoxycarbonyl and napthyloxycarbonyl; aryloxyalkanoyl such as phenoxyacetyl and phenoxypropionyl; arylcarbamoyl such as phenylcarbamoyl; arylthiocarbamoyl such as phenylthiocarbamoyl; arylglyoxyloyl such as phenylglyoxyloyl and naphthylglyoxyloyl; arylsulfonyl such as phenylsulfonyl and napthylsulfonyl; heterocycliccarbonyl; heterocyclicalkanoyl such as thienylacetyl, thienylpropanoyl, thienylbutanoyl,

thienylpentanoyl, thienylhexanoyl, thiazolylacetyl, thiadiazolylacetyl and tetrazolylacetyl; heterocyclicalkenoyl such as heterocyclicpropenoyl, heterocyclicbutenoyl, heterocyclicpentenoyl and heterocyclichexenoyl; and heterocyclicglyoxyloyl such as thiazolylglyoxyloyl and thienylglyoxyloyl.

In alternative embodiments, the spacer molecule may have a branched structure whereby multiple functional groups may be attached at the ends of the branches.

Generally, there are two ways in which the spacer S may be incorporated into the synthon:

(1) A spacer molecule with a desired functional group already attached to at least one end is chemically coupled to the backbone.

(2) A spacer molecule is attached to the active constituent. Then in a separate synthetic step, the spacer molecule is further modified to attach a desired functional group.

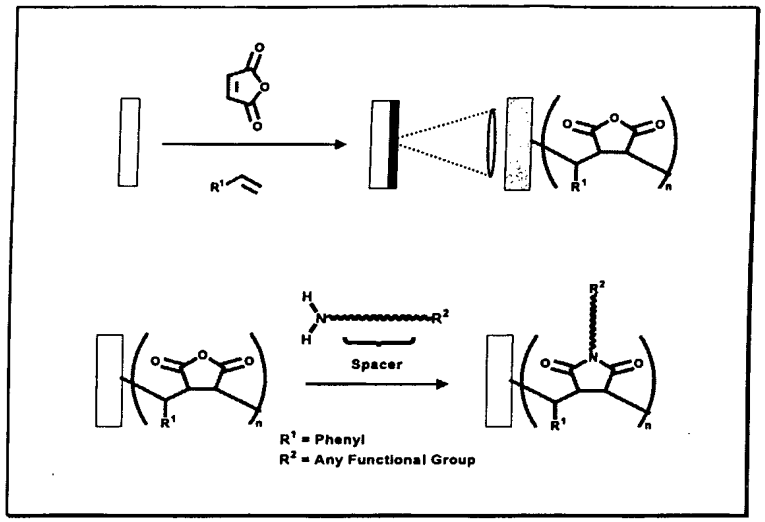
In some embodiments, a spacer molecule may be attached, then modified with more than one functional group.

In one embodiment the spacer molecule is a linear chain molecule and a functional tether is formed by modifying the end of the chain distal from the site of attachment to the active constituent of the synthon.

By modifying the chemical or structural properties of the spacer molecule it is possible to generate synthons with a range of macroscopic coating properties. For example, glycol oligomer chains provide a relatively rigid linear structure, whereas simple hydrocarbons adopt more folded conformations. These differences in spacer geometry also may vary with chain length or the presence of charged groups in the spacer molecule. These differences in geometry provided by the spacer molecule properties directly affects the orientation of the functional group with respect to the backbone and thereby affects the overall macroscopic properties of the surface coating. Modification of these properties may greatly affect the complementary or antagonistic interactions between the surface and a biomolecule, cell or other chemical entity immobilized thereon.

Scheme 3 below illustrates the formation of a backbone coating on a substrate and subsequent attachment of a spacer.

Scheme 3



In Scheme 3, the backbone coating is applied by polymerization of the active constituent, maleic anhydride, and the passive constituent, styrene. The spacer unit features an amine at one end that forms a covalent linkage to the active constituent resulting in a maleimide.

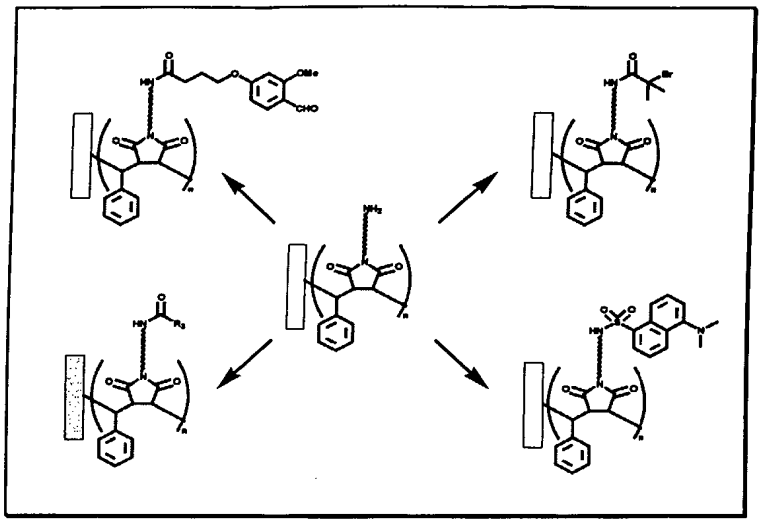
Preferably the spacer unit is a residue of a diamine, more preferably an alkyl diamine. It is particularly preferred that the spacer unit S is a residue of 1.5-diaminopentane or N-(3-aminopropyl)-1,3-propanediamine.

The Functional Group

The functional group may serve different roles in various embodiments. For example, the functional group may act as a site for further chemical modification of the surface. In the instance, where the functional group is capped with a polymerization initiator, the possibility exists to add another level of synthon diversity.

In Scheme 4 below, a spacer with an amine moiety provides the site for chemical modification with four different functional groups thereby resulting in four different, but related synthon surface coatings.

Scheme 4



Preferably, the functional group F is a group capable of binding or chemically reacting with a biological molecule or component. The functional group F also preferably comprises a primary or secondary amine group.

Screening for Surface Optimization

In scheme 4, the functional group on each of the four coatings may serve as the primary site for a complementary binding interaction. By screening the four coatings in a desired solid phase binding assay, one may determine which surface is optimal. Subsequently, based on the best of the four synthons shown in Scheme 4, new libraries of related synthons may be generated to further optimize the surface for the desired application in an iterative fashion. For example, the next iteration may vary only the spacer length. Hence, synthons may be generated with functional groups exhibiting a range of molecular diversity in order to find the optimal surface for binding a complementary molecular species such as a receptor or other large biomolecule. For example, a library of synthons may be generated comprising a range of functional groups in order to find the optimal surface coating for binding the β-adrenergic receptor in a surface plasmon resonance experiment.

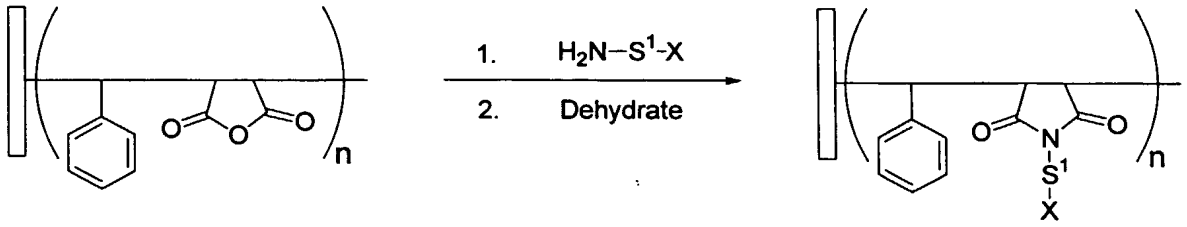
High-Throughput Advantage

Morever, Scheme 4 illustrates the high-throughput advantage afforded by some embodiments of the synthon-based approached. As mentioned in the Background of the Invention, generation of surface diversity on solid phases has been limited by the difficulty of developing chemical methods for grafting new coatings onto solid substrates. Prior methods have focused on utilizing solution reactions to generate a diverse library of candidate compounds for coating a substrate. These methods have encountered a bottleneck in getting the solution-phase compounds coated onto a solid-phase substrate. This bottleneck results from the general lack of development of the science of grafting materials onto solids to form coatings.

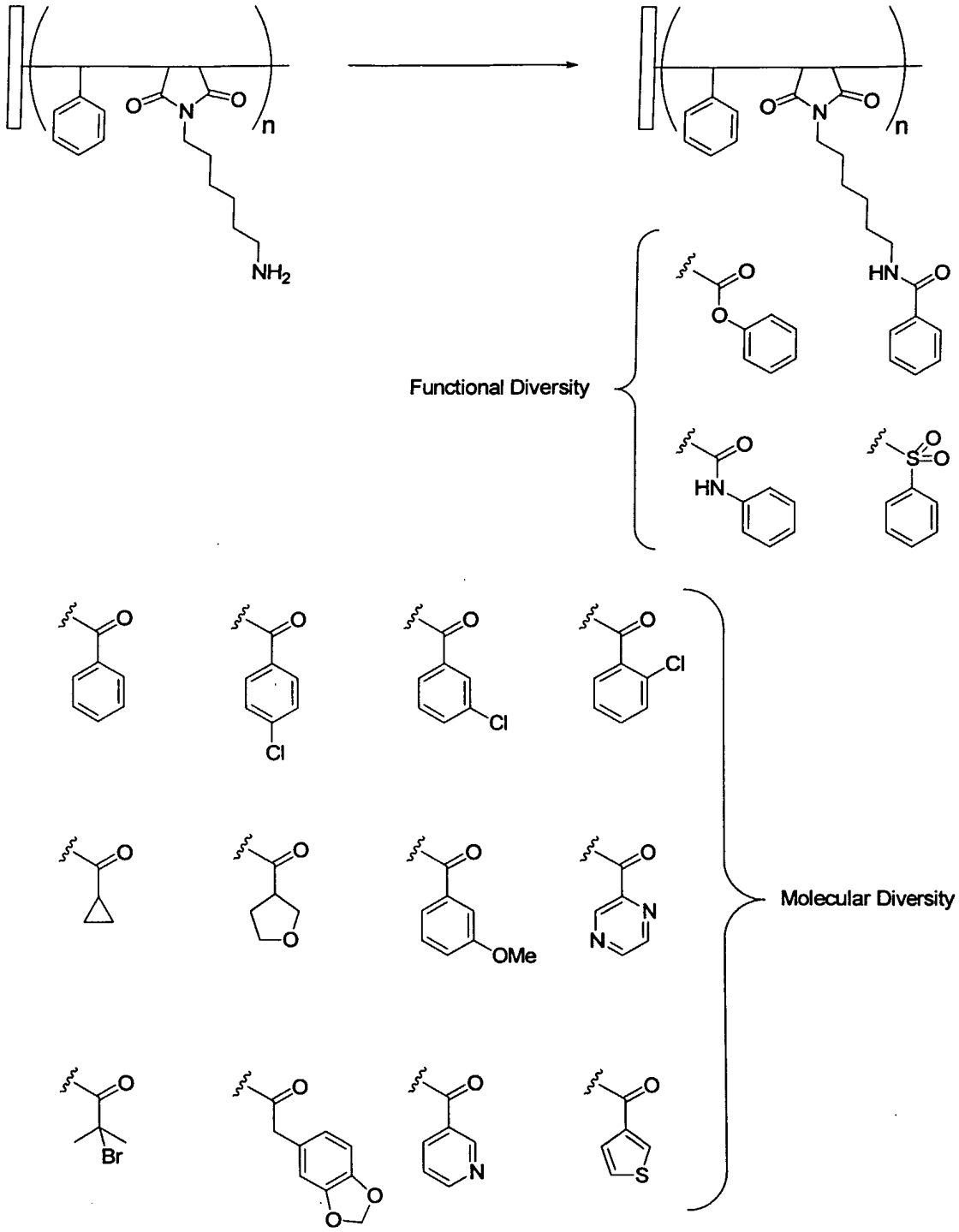
As shown in Scheme 4, the present invention provides a high-throughput solution to generating surface diversity by avoiding this bottleneck. Instead, in preferred embodiments, libraries of diverse surfaces may be generated from a single backbone coating applied by a well-characterized grafting procedure. Subsequently, diversity may be introduced to the solid phase surface in a combinatorial manner by varying the spacer and functional groups structures through well-known synthetic routes.

High-throughput generation of molecular diversity for detecting complementary binding interactions, as well as, for further chemical modification may be achieved by modifying the functional group on a relatively simple synthon backbone-spacer configuration. As shown in Schemes 5 and 6 below, when H_2N-S^1-X is a symmetrical diamine such as H_2N-$(CH_2)_6$- NH_2, a large number of functional groups with a range of functional and molecular diversity may be added.

Scheme 5



Scheme 6


n
NH2
Functional Diversity
HN
HN
Cl
Cl
Cl
OMe
N
N
Br
N
S
Molecular Diversity

Incorporation of Grafting and Polymerization Methods

In a preferred embodiment, the synthon-based approach to generation of diverse surface coatings may be carried out using well-known or readily-constructed free radical polymerization technology. This embodiment is particularly well-suited to generating synthon surface coatings on polymeric substrates such as polyolefins. In preferred embodiments, the polymeric substrate such as polypropylene or , may be already be coated with sytreneic, (meth)acrylic, (meth)acrylamides, or other related graft coatings. The manner by which this initial coating is a generated is well known in the art, gamma grafting, where by the initiation requirements for the graft polymerisation to occur is from a cobalt-60 source, or the like.

The Substrate

The combinatorial advantages of the present synthon-based surface discovery system are independent of the nature of the base substrate material or how the synthon is applied to the surface. Hence surface diversity may be explored across a wide range of substrates. The substrate used in accordance with present invention is generally a solid and provides an integral surface or plurality of surfaces upon which the different surface coating(s) may be applied. Preferably, the substrate is selected from glass, silicon, metals, and organic polymers, other synthetic or natural materials, and combinations thereof.

The substrate may for example be provided in the form of a microscope slide, microtitre plate, porous membrane, pipette tip, tube or a plurality of beads.

Preferably, the substrate is an organic polymer. Suitable organic polymers include, but are not limited to, polytetrafluoroethylene, polystyrene, polypropylene, polyethylene, polyvinylidenefluoride and polymethylmethacrylate.

Further, the substrate may be porous, non-porous, and/or any geometric shape, e.g. bead, or flat. A variety of porous polymeric substrates with co-continuous architecture useful with the present invention are described in co-pending US patent application no. 10/052,907 filed January 17, 2002, which is hereby incorporated by reference herein.

In a preferred embodiment of the invention the substrate is an organic polymer in the form of a plurality of beads. Preferably, the beads are labelled such that a particular coating can be related to a particular bead or subgroup of beads. Suitable polymeric beads

for use as a substrate in accordance with the present invention include, but is not limited to, Luminex™ beads.

Multiplexed Applications

The present compositions and methods allow surface diversity to be explored in a high-throughput fashion by, for example, building different synthons in an array format on a single substrate. A variety of multiplex formats such as arrays or beads may be used. For example, a single synthon backbone coating may be applied across the full substrate surface. Then different spacer units or functional group variants may be generated in different localized regions on the substrate.

As used herein, a "region" of a substrate includes a point, area or other location on the surface of the substrate. Each different surface coated on the substrate occupies discrete regions on the substrate.

In one preferred embodiment, photolithographic or micromirror methods may be used to spatially direct light-induced chemical modifications of spacer units or functional groups resulting in attachment at specific localized regions on the surface of the substrate. Light-directed methods of controlling reactivity and immobilizing chemical compounds on solid substrates are well-known in the art and described in U.S. Patent Nos. 4,562,157, 5,143,854, 5,556,961, 5,968,740, and 6,153,744, and PCT publication WO 99/42813, each of which is hereby incorporated by reference herein.

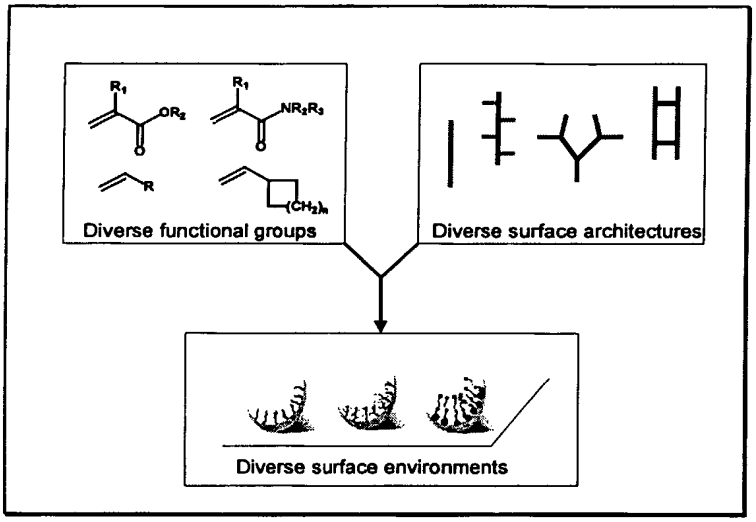
Alternatively, plural localized synthon generation on a single substrate may be achieve by precise deposition of chemical reagents. Methods for achieving high spatial resolution in depositing small volumes of a liquid reagent on a solid substrate are disclosed in U.S Patent Nos. 5,474,796 and 5,807,522, both of which are hereby incorporated by reference herein.

The term "array" may or may not require the identification of each different surface coating in terms of co-ordinates for its location. An array may be in a pattern or be random and may comprise two or more coatings, or the same coating in different regions on the same substrate. The underlying substrate may be uniform in its ability to accept a surface coating. Or the substrate may have regions with different abilities to bind specific surface coatings resulting in a spatial pattern depending on the coating.

Screening of Diverse Surface Environments

Surface coatings prepared using the synthon-based approach of the present invention may find use in a wide range of solid phase applications. The generation of a combinatorial selection of surface coatings provides a spectrum of molecular and macroscopic surface properties. The method provides a diversity of surface environments as shown in Scheme 7 below:

Scheme 7



Each of these surfaces may potentially create an optimum environment or have optimal properties for a particular solid phase application. However, the greater the number of diverse surfaces in a library requires more screening for each particular application.

Generally, the surface coatings of the present invention may be screened for optimal performance in a solid phase application of interest by methods well known in the art. For example, such screening may involve detecting specific binding of cells to the surface and consequently may utilize flow cytometry as, for example, described by Needels *et al.* (1993).

Other screening methods useful with the present invention include any of the great number of isotopic and non-isotopic labeling and detection methods well-known in the chemical and biochemical assay art. For example, a library of surface coatings of the present invention may be screened for the ability to bind a specific peptide in an active

configuration on the surface. An active configuration refers to an orientation of the molecule on the surface coating whereby the molecule may be specifically detected with a selected probe molecule, e.g. a fluorescently coupled antibody that specifically binds the molecule.

Alternatively, spectroscopic methods well-known in the art may be used to determine directly whether a molecule is bound to a surface coating in an desired configuration. Spectroscopic methods include e.g., UV-VIS, NMR, EPR, IR, Raman, mass spectrometry and other methods adapted to surface analysis well-known in the art.

Examples of biological compounds that may be screened for binding in the proper configuration on surface coating generated by the synthon-based approach of the present invention include, e.g. agonists and antagonists for cell membrane receptors, toxins, venoms, viral epitopes, hormones, sugars, cofactors, peptides, enzyme substrates, drugs inclusive of opiates and steroids, proteins including antibodies, monoclonal antibodies, antisera reactive with specific antigenic determinants, nucleic acids, lectins, polysaccharides, cellular membranes and organelles.

In addition, the present invention may be employed to generate optimal surface coatings for immobilized nucleic acids. These coatings may be used in any of a large number of well-known hybridization assays where nucleic acids are immobilized on a surface of a substrate, e.g. genotyping, polymorphism detection, gene expression analysis, fingerprinting, and other methods of DNA- or RNA-based sample analysis or diagnosis.

Various aspects of the present invention may be conducted in an automated or semi-automated manner, generally with the assistance of well-known data processing methods. Computer programs and other data processing methods well known in the art may be used to store information including e.g. surface coating library chemical and macroscopic properties. Data processing methods well known in the art may be used to read input data covering the desired characteristics.

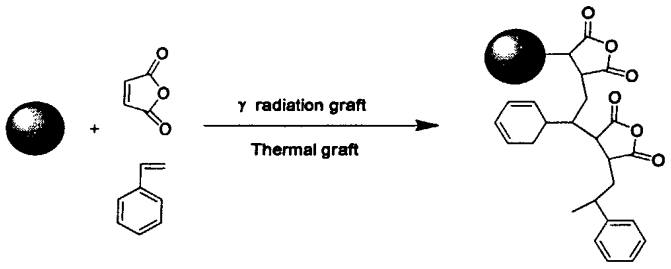
Alternatively, or in addition, data processing methods well known in the art may be used to control the processes involved in the present invention, including e.g applying or polymerizing the backbone coating on the substrate; control of chemical reactions involved in further generating the synthon; and/or the reactions and interactions occurring in, within or between a population or array of surface coatings on a substrate.

The invention will now be described with reference to non-limiting examples. However it is to be understood that the particularity of the following description of the invention is not to supersede the generality of the invention previously described.

EXAMPLES

1. ***Generation of a maleic anhydride (MAn)-styrene backbone coating on a polymeric solid substrate.***

Scheme 8

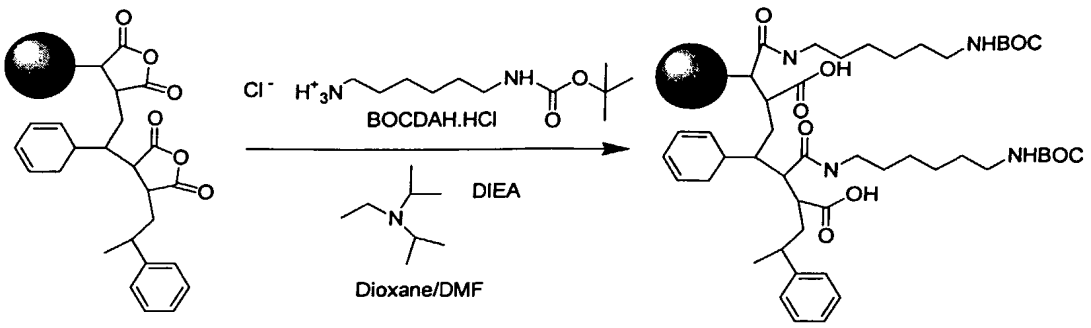


Scheme 8, above, illustrates the reaction carried out in generating the backbone coating. Plastic hollow cylinders, measuring 6mm in length, 3 mm in diameter were pre-radiated in air at room temperature (1.8 KGy/h for 7 hours). A 40% (v/v) solution of styrene and maleic anhydride, present in mole equivalent proportions, in toluene was prepared and the added to the irradiated plastic cylinders. The mixture was then purge with nitrogen gas for 5 minutes via a septum and heated, with agitation at 60 C for 6 hours. The plastic cylinders were then isolated from the polymerised solution, washed thoroughly to remove non-grafted polymer and dried to constant weight.

2. ***Attachment and subsequent deprotection of tert-butyl carbamate (BOC) protected diamines spacer units to MAn-Sty backbone coating.***

Step 1: Ring Opening with Amines (see Scheme 9, below)

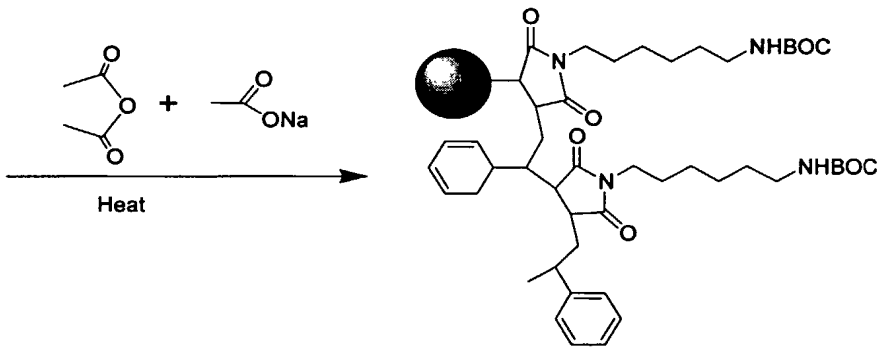
Scheme 9



A 1:1 DMF / Dioxane solution comprising an excess equivalents of the protected diamine was charged with plastic cylinders prepared above in example 1. A 6x excess DIEA was added to the solution and the solution left to react at 60C for 2 hours, after which the plastic cylinders where isolated from the reaction mixture and washed thoroughly. Spectroscopic evidence (ATR and Raman) established the disappearance of the anhydride.

Step 2: Ring closure to the Imide (see Scheme 10, below)

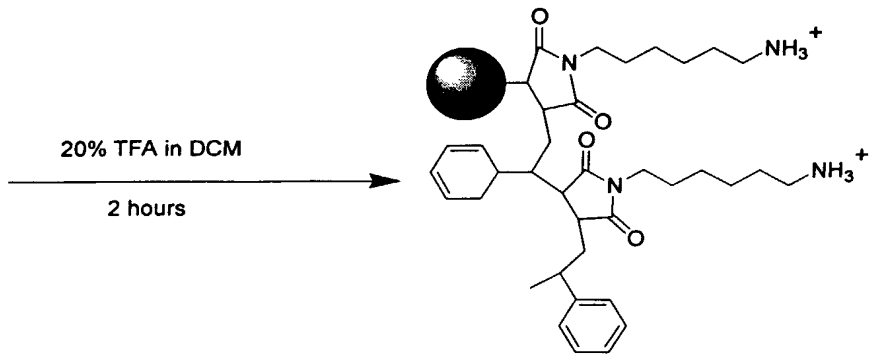
Scheme 10



The ring closure of the amic acid was effected by heating the material from step 1 of example 2 prepared above, at 60C in DMF in the presence of acetic anhydride and sodium acetate for 4 hours. The plastic cylinders were then washed extensively to afford the ring closed, grafted imide.

Step 3: Liberating the Amine (see Scheme 11, below)

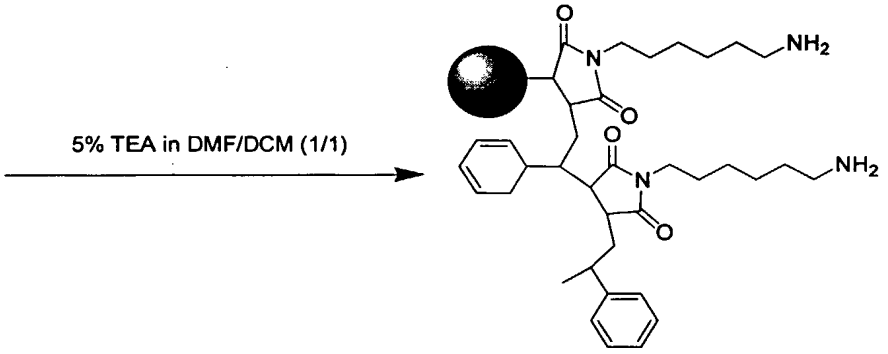
Scheme 11



The removal of the amine protection group was performed under standard acid deprotection conditions by placing a sample of the plastic cylinders prepared above in example 2, step 2 were placed in a 20% Trifluoroacetic acid in dichloromethane for 2 hours. The deprotected, acidified samples were than washed extensively with dichloromethane prior to neutralization.

Step 4: Neutralization of grafted amine (see Scheme 12, below)

Scheme 12



The acidified samples prepared above in example 2, step 3 were treated with 5% triethyl amine in a 1:1 dimethyl formamide / dichloromethane, for 20 minutes, then washed extensively with dimethyl formamide and dichloromethane, prior to drying and determination of amine activity as described in Example 3, below.

3. Determination of Amine Activity.

A sample of the grafted material prepared above in example 2, step 4, were treated with an excess of Fmoc-β-Ala-OH in dichloromethane, in the presence of diisopropyl carbodiimide. The Fmoc from the coupled Fmoc-β-Ala-OH to the pendant amine on the

plastic cylinder was then cleaved by exposure of the plastic cylinders to a 20% solution piperidine in dimethyl formamide and the liberated Fmoc detected spectrophotometrically, to afford a concentration of active amines on the graft of 0.108 micromoles.

4. Synthon Coating: Disks Examples

I. Library of Maleimides

Step 1. Preparation of Maleic Anhydride/Styrene Graft Co-polymer on PFA disks. Maleic anhydride/Styrene was covalently attached onto a tetrafluoroethylene-perfluoroalkyl-vinylether copolymer (PFA) disk using the γ-irradition technique. Three thousand PFA disks (6 mm diameter x 0.8 mm thickness) were immersed in 150 mL 20% maleic anhydride in ethyl acetate (w/v) and 150 mL 20% styrene in ethyl acetate (v/v) containing 0.010 M HCl in dioxane in a 500 mL glass bottle. The solution was degassed by bubbling with $N_{2(g)}$ for 10 min. The glass bottle was sealed with a Teflon screw cap and γ-irradiated with a ^{60}Co source. The grafted disks were thoroughly washed with DMF and CH_2Cl_2 to remove residual monomer and non-grafted co-polymer and dried overnight under vacuum at 30°C. After drying, the disks were weighed to give an average mass change of 0.92% per disk (1.94 $\mu g/mm^2$).

Step 2: Reaction of Maleic Anhydride/Styrene Graft System with Primary Amines. A 50 mL glass vial was charged with maleic anhydride/styrene grafted PFA disks (100 disks) and 20 mL of primary amine (1 M, Table 3) in DMF before the vial was sealed and shaken overnight. After 16 h, the solution was removed and the disks washed with DMF and CH_2Cl_2 before drying under vacuum to give the mixed (amide-carboxylic acid-phenyl) system.

Table 3. List of Primary Amines for Disks

No.	Amine	No.	Amine
1	2-(Aminomethyl)-18-crown-6	25	CYCLOHEXANEMETHYLAMINE
2	4-METHOXYPHENETHYLAMINE	26	5-AMINO-1-PENTANOL
3	Benzylamine	27	ISOPentylamine

4	N-Acetylethylenediamine	28	1-(3-AMINOPROPYL)IMIDAZOLE
5	Undecyclamine	29	2-Methoxyethylamine
6	1-NAPHTHALENEMETHYLAMINE	30	Ethanol amine
7	1-(2-AMINOETHYL)PYRROLIDINE	31	3-Aminopropionitrile
8	2-(2-Aminoethoxy)ethanol	32	3-Methoxypropylamine
9	Tetrahydrofurfuryl amine	33	3-FLUOROBENZYLAMINE
10	2-(2-CHLOROPHENYL)ETHYLAMINE	34	3,4,5-Trimethoxybenzylamine
11	Propylamine	35	4-Methoxybenzylamine
12	2-(aminomethyl)pyridine	36	2-Amino-1-propene-1,1,3-tricarbonitrile
13	3,4-DIMETHOXYPHENETHYLAMINE	37	p-Aminophenyl-beta-D-glucopyranoside
14	3-PHENYL-1-PROPYLAMINE	38	D-Glucosamine hydrochloride
15	4-CHLOROBENZYLAMINE	39	p-Aminophenyl-beta-D-galactopyranoside
16	1-(2-AMINOETHYL)PIPERIDINE	40	Bis-homotris
17	4-PHENYLBUTYLAMINE	41	3-(Diethylamino)propylamine
18	4-AMINO-1-BUTANOL	42	2-METHOXYBENZYLAMINE
19	4-FLUOROBENZYLAMINE	43	Isobutylamine
20	6-AMINO-1-HEXANOL	44	BUTYLAMINE
21	DECYLAMINE	45	4-(TRIFLUOROMETHYL)BENZYLAMINE
22	NONYLAMINE	46	3,5-DIMETHOXYBENZYLAMINE
23	Octylamine	47	3-FLUOROPHENETHYLAMINE
24	VERATRYLAMINE	48	Pentylamine

Step 3: Cyclization of Mixed System to give Styrene/Maleimide Graft Co-polymer. Mixed amide-carboxylic acid-styrene PFA disks (50 disks) derived from primary amines were treated with toluene (50 mL), acetic anhydride (0.25 M), and sodium acetate (0.025 M) before heating to 80°C overnight. After 16 h, the vial was drained of reagent and the disks washed with toluene, DMF, and then CH_2Cl_2 before drying under vacuum to afford the library of styrene/maleimide surfaces, generated from one initial surface.

At each stage in the coating assembly, XPS and ATR spectra were acquired and indicated that each transformation had been performed. Further, the assembled library of maleimides on disks was screened against anti Rabbit IgG, and a spectrum of very low to very high protein bindings events were observed.

II. Library of Maleimides with Diamine Spacers and Capping Groups

Step 1. Preparation of Maleic Anhydride/Styrene Graft Co-polymer on PFA disks. Maleic anhydride/Styrene was covalently attached onto a tetrafluoroethylene-perfluoroalkyl-vinylether copolymer (PFA) disk using the γ-irradition technique. Three thousand PFA disks (6 mm diameter x 0.8 mm thickness) were immersed in 150 mL 20% maleic anhydride in ethyl acetate (w/v) and 150 mL 20% styrene in ethyl acetate (v/v) containing 0.010 M HCl in dioxane in a 500 mL glass bottle. The solution was degassed by bubbling with $N_{2(g)}$ for 10 min. The glass bottle was sealed with a Teflon screw cap and γ-irradiated with a ^{60}Co source. The grafted disks were thoroughly washed with DMF and CH_2Cl_2 to remove residual monomer and non-grafted co-polymer and dried overnight under vacuum at 30°C. After drying, the disks were weighed to give an average mass change of 0.92% per disk (1.94 $\mu g/mm^2$).

Step 2: Reaction of Maleic Anhydride/Styrene Graft System with Diamines on Disk. 1943 PFA discs grafted with maleic anhydride/styrene from Step 1 were then split into 29 batches of 67 discs. Each batch was treated with a different diamine (0.5 M in DMF) from Table 4 to give, after washing, 29 different mixed (amide-carboxylic acid-phenyl) intermediates containing free amines.

Table 4. List of Diamine Spacers for Maleimide Library

No.	Diamine	No.	Diamine
1	Ethylenediamine	16	Pentaethylenehexamine
2	1,4-Diaminobutane	17	1,4-Bis(3-aminopropyl)piperazine
3	1,12-Diaminododecane	18	2,2'-Oxybis(ethylamine) dihydrochloride
4	1,5-Diaminopentane	19	3,3'-Diamino-*N*-methyldipropylamine
5	1,3-Diaminopropane	20	2,2'-Dimethyl-1,3-diaminopropane

6	Diethylenetriamine	21	*N,N'*-Bis(2-aminoethyl)-1,3-propanediamine
7	Dipropylenetriamine	22	2,2'-(Ethylenedioxy)bis(ethylamine)
8	Tetraethylenepentamine	23	DAB((PA)4 Generation 1.0
9	Triethylenetetramine	24	DAB((PA)4 Generation 2.0
10	1,3-Cyclohexanebis(methylamine)	25	*p*-Xylylenediamine
11	1,9-Diaminononane	26	O,O'-Bis(3-aminopropyl)polyethylenediamine
12	4,9-Dioxa-1,12-dodecanediamine	27	Polyethylenimine
13	*N,N'*-Bis(3-aminopropyl)ethylenediamine	28	1,7-Diaminoheptane
14	Bis(hexamethylene)triamine	29	4,7,10-Trioxa-1,13-trideanediamine
15	Tris(2-aminoethyl)amine		

Step 3: Reaction of Mixed (Amide-carboxylic acid-phenyl) Amine Intermediates with Carboxylic Acids.

Each batch of diamines from step 2 was split into 67 different separate discs. Each disc was treated with a different carboxylic acid from Table 5 in a

Table 5. List of Carboxylic Acid Capping Groups for Maleimide Library

No.	Acid	No.	Acid
1	BOC-3-(1-naphthyl)-L-alanine	35	2-Norbornane acetic acid
2	N(alpha)-BOC-L-lysine (Fmoc)	36	2,3,4-Trimethoxybenzoic acid
3	D-Tyrosine	37	2-HYDROXY-1-NAPHTHOIC ACID
4	O-tert-Butyl-L-serine (Fmoc)	38	4-TERT-BUTYLCYCLOHEXANECARBOXYLIC ACID
5	FMOC-L-glutamic acid 5-benzyl ester	39	2-Thiopheneacetic acid
6	D-Phenylalanine (BOC)	40	2-Biphenylcarboxylic acid
7	BOC-L-Tyrosine	41	3,4-Diaminobenzoic acid
8	L-Tyrosine (BOC)	42	DIETHYLPHOSPHONOACETIC ACID

9	N-Benzyloxycarbonyl-L-tyrosine	43	Flufenamic acid
10	FMOC-L-Phenylalanine	44	TRIDECANOIC ACID
11	N-(9-FLUORENYLMETHOXYCARBONYL)-L-PROLINE	45	(1R,3R,4R,5R)-(-)-QUINIC ACID
12	N-alpha-Carbobenzyloxy-L-tryptophan	46	2,2-Bis(hydroxymethyl)propionic acid
13	N-CBZ-L-METHIONINE	47	p-Toloyl chloride
14	N-FMOC-(L-ALANINE-OH)-H2O	48	Propionic anhydride
15	N-Carbobenzyloxy-L-proline	49	3-Mercaptopropionic acid
16	2-(DIPHENYLPHOSPHINO)BENZOIC ACID	50	Gibberellic acid
17	1-Pyrenebutyric acid	51	Z-L-leucyl-L-alanine
18	(1S)-(-)-CAMPHANIC chloride	52	R(+)-N-(alpha-Methylbenzyl)phthalic acid monoamide
19	2,3,4,5-Tetrafluorobenzoyl chloride	53	(+)-mono-(1S)-Menthyl phthalate
20	Docosanoic acid	54	R(-)-2-Oxothiazolidine-4-carboxylic acid
21	2,6-Difluorophenylacetic acid	55	9H-Fluorene-9-carboxylic acid
22	Piperonyloyl chloride	56	Orotic acid anhydrous
23	2,3,4-TRIHYDROXYBENZOIC ACID	57	BOC-L-leucine
24	Pentafluorobenzoyl chloride	58	15-Hydroxypentadecanoic acid
25	4-METHOXYCYCLOHEXANECARBOXYLIC ACID	59	ACEMETACIN
26	3-Iodo-4-methylbenzoic acid	60	N-T-BOC-S-TRITYL-L-CYSTEINE
27	4-Octyloxybenzoic acid	61	URACIL-4-ACETIC ACID
28	Cyanoacetic acid	62	(+/-)-4-METHYLOCTANOIC ACID
29	2-METHYL-1-CYCLOHEXANECARBOXYLIC ACID	63	N-ALPHA-T-BOC-NEPSILON-CBZ-L-LYSINE
30	N-TRITYLGLYCINE	64	Indomethacin
31	3-Phenoxybenzoic acid	65	N-BENZOYL-BETA-ALANINE
32	3-Indolebutyric acid	66	N-ACETYL-L-TRYPTOPHAN
33	3,5-Diisopropylsalicylic acid	67	MEFENAMIC ACID
34	4-Methylvaleric acid		

solution of DMF, 1-hydroxy-7-azabenztriazole (0.25 M), and diisopropylethylamine (0.5 M). The reaction was agitated overnight before washing with DMF and methylene chloride to remove excess reagent.

Step 4: Cyclization of Mixed System to give Styrene/Maleimide Graft Co-polymer. Mixed amide-carboxylic acid-styrene PFA disks from *step 3* (50 disks) were treated with acetic anhydride (0.25 M) and sodium acetate (0.025 M) in toluene before heating to 80°C overnight. After 16 h, the vial was drained of reagent and the disks washed with toluene, DMF, and then CH_2Cl_2 before drying under vacuum to afford the library of styrene/maleimide surfaces, generated from one initial surface.

At each stage in the coating assembly, XPS and ATR spectra were acquired and indicated that each transformation had been performed. Further, the assemble library of maleimides with diamine spacers and dapping groups on disks was screened against anti Rabbit IgG, and a spectrum of very low to very high protein bindings were observed.

5. Synthon Coating: Microarray Examples

I. Library of Maleimides

Step 1. Preparation of Maleic Anhydride/Styrene Graft Co-polymer on microscope slide. A procedure for applying a Synthon Coating in a microarray format can be accomplished as follows: A microscope slide of dimensions 2.5 x 7.5 x 0.1 cm, prepared from the injection molding of tetrafluoroethylene-perfluoroalkyl-vinylether copolymer (PFA), can be masked to create an array of 16 x 250 um circular spots. Treatment of the masked slide with heptane plasma (5 min, 20 W, 10^{-3} torr) followed by removal of the mask yields a PFA slide consisting of 16 x 250 um thinly coated heptane spots. UV irradiation of the slide in the presence of benzophenone (0.05 M) in methanol followed by simultaneous polymerization and grafting of maleic anhydride (1.75 M) and styrene (1.75 M) in ethyl acetate selectively derivatizes the heptane layer to give arrayed spots that are densely functionalised with anhydride groups.

Step 2: Reaction of Maleic Anhydride/Styrene Graft Slide with Primary Amines.

Primary amine containing compounds (0.5 M) dissolved in DMF readily attach to the surface upon robotic printing of nanolitre droplets to each spot *via* ring opening of the anhydride. Each spot of 3 slides from step 1 were treated with a different primary amine (Table 6) to give three microarrays of 16 different mixed (amide-carboxylic acid-phenyl) intermediates. The arrays were washed exhaustively with DMF, CH_2Cl_2, and 1% acetic acid in DMF before drying under vacuum.

Table 6. List of Primary Amines for Microarray

No.	Amine	No.	Amine
1	2-(Aminomethyl)-18-crown-6	25	CYCLOHEXANEMETHYLAMINE
2	4-METHOXYPHENETHYLAMINE	26	5-AMINO-1-PENTANOL
3	Benzylamine	27	ISOPentylamine
4	N-Acetylethylenediamine	28	1-(3-AMINOPROPYL)IMIDAZOLE
5	Undecyclamine	29	2-Methoxyethylamine
6	1-NAPHTHALENEMETHYLAMINE	30	Ethanol amine
7	1-(2-AMINOETHYL)PYRROLIDINE	31	3-Aminopropionitrile
8	2-(2-Aminoethoxy)ethanol	32	3-Methoxypropylamine
9	Tetrahydrofurfuryl amine	33	3-FLUOROBENZYLAMINE
10	2-(2-CHLOROPHENYL)ETHYLAMINE	34	3,4,5-Trimethoxybenzylamine
11	Propylamine	35	4-Methoxybenzylamine
12	2-(aminomethyl)pyridine	36	2-Amino-1-propene-1,1,3-tricarbonitrile
13	3,4-DIMETHOXYPHENETHYLAMINE	37	p-Aminophenyl-beta-D-glucopyranoside
14	3-PHENYL-1-PROPYLAMINE	38	D-Glucosamine hydrochloride
15	4-CHLOROBENZYLAMINE	39	p-Aminophenyl-beta-D-galactopyranoside
16	1-(2-AMINOETHYL)PIPERIDINE	40	Bis-homotris
17	4-PHENYLBUTYLAMINE	41	3-(Diethylamino)propylamine
18	4-AMINO-1-BUTANOL	42	2-METHOXYBENZYLAMINE
19	4-FLUOROBENZYLAMINE	43	Isobutylamine
20	6-AMINO-1-HEXANOL	44	BUTYLAMINE
21	DECYLAMINE	45	4-(TRIFLUOROMETHYL)BENZYLAMINE
22	NONYLAMINE	46	3,5-DIMETHOXYBENZYLAMINE
23	Octylamine	47	3-FLUOROPHENETHYLAMINE
24	VERATRYLAMINE	48	Pentylamine

Step 3: Cyclization of Mixed System to give Styrene/Maleimide Graft Co-polymer. Subsequent dehydration of the entire array using acetic anhydride (0.25 M) and sodium acetate (0.025 M) at 80°C in toluene gives arrays of 16 different surface bound maleimides/styrene co-polymers.

At each stage in the coating assembly, XPS and ATR spectra were acquired and indicated that each transformation had been performed. Further, the assemble library of maleimides on a microarray was screened against anti Rabbit IgG, and a spectrum of very low to very high protein bindings events were observed.

II. Library of Mixed (amide-carboxylic acid-phenyl) Systems from Secondary Amines on Microarray.

Step 1. Preparation of Maleic Anhydride/Styrene Graft Co-polymer on microscope slide. A procedure for applying a Synthon Coating in a microarray format can be accomplished as follows: A microscope slide of dimensions 2.5 x 7.5 x 0.1 cm, prepared from the injection molding of tetrafluoroethylene-perfluoroalkyl-vinylether copolymer (PFA), can be masked to create an array of 16 x 250 um circular spots. Treatment of the masked slide with heptane plasma (5 min, 20 W, 10^{-3} torr) followed by removal of the mask yields a PFA slide consisting of 16 x 250 um thinly coated heptane spots. UV irradiation of the slide in the presence of benzophenone (0.05 M) in methanol followed by simultaneous polymerization and grafting of maleic anhydride (1.75 M) and styrene (1.75 M) in ethyl acetate selectively derivatizes the heptane layer to give arrayed spots that are densely functionalised with anhydride groups.

Step 2: Reaction of Maleic Anhydride/Styrene Graft Slide with Secondary Amines. A PFA slide grafted with 16 maleic anhydride/styrene spots was elaborated with 16 different secondary amines (0.5 M, Table 7) dissolved in DMF via robotic printing. Washing of the slide with dimethylformamide followed by 1% acetic acid in dimethylformamide gives 16 x 250 um different mixed (amide-carboxylic acid-styrene) spots on the PFA slide.

Table 7. List of Secondary Amines for Microarray

No.	Secondary Amine	No.	Secondary Amine
1	Dimethylamine	9	4-Piperidinone monohydrate hydrochloride
2	3,3-Iminodipropionitrile	10	1-Acetylpiperazine
3	Morpholine	11	1,2,3,4-Tetrahydroisoquinoline
4	Bis(2-methoxyethyl)amine	12	Pyrrolidinone
5	Piperidine	13	*N*-Methylpropargyl amine
6	Diethyl amine	14	N, N, N'-Trimethylethylenedianine
7	*N*-Benzylmethylamine	15	Thiomorpholine
8	1-Methylpiperazine	16	Nipecotamide

At each stage in the coating assembly, XPS and ATR spectra were acquired and indicated that each transformation had been performed. Further, the assemble library of mixed (amide-carboxylic acid-phenyl) systems from secondary amines on microarray was screened against anti Rabbit IgG, and a spectrum of very low to very high protein bindings events were observed.

III. Library of Mixed (amide-amide-phenyl) System on Microscope Slide

Step 1. Preparation of Maleic Anhydride/Styrene Graft Co-polymer on microscope slide. A procedure for applying a Synthon Coating in a microarray format can be accomplished as follows: A microscope slide of dimensions 2.5 x 7.5 x 0.1 cm, prepared from the injection molding of tetrafluoroethylene-perfluoroalkyl-vinylether copolymer (PFA), can be masked to create an array of 16 x 250 um circular spots. Treatment of the masked slide with heptane plasma (5 min, 20 W, 10^{-3} torr) followed by removal of the mask yields a PFA slide consisting of 16 x 250 um thinly coated heptane spots. UV irradiation of the slide in the presence of benzophenone (0.05 M) in methanol followed by simultaneous polymerization and grafting of maleic anhydride (1.75 M) and styrene (1.75 M) in ethyl acetate selectively derivatizes the heptane layer to give arrayed spots that are densely functionalised with anhydride groups.

Step 2: Reaction of Maleic Anhydride/Styrene Graft Slide with Secondary Amines.

A PFA slide grafted with 16 maleic anhydride/styrene spots was elaborated with 16 different secondary amines (0.5 M, Table 7 above) dissolved in DMF via robotic printing. Washing of the slide with dimethylformamide followed by 1% acetic acid in dimethylformamide gives 16 x 250 um different mixed (amide-carboxylic acid-styrene) spots on the PFA slide.

Step 3: Reaction of Mixed (amide-carboxylic acid-phenyl) System with Diamine.

Twenty-nine copies of the slide in step 2 were treated with DMAP (10 mol %), 1,3-diisopropyl carbodiimide (0.25 M), and *N*-hydroxysuccinimide (0.15M) in DMF. After washing with DMF, the slides were separated and each treated with a different diamine from Table 6 above. After several hours, the slides were washed with DMF and allowed to dry under vacuum to give microarrays of mixed (2°-Amide-1°-amide-phenyl)amine systems. Hence, all slides contain the same 16 secondary amines, one for each spot, but each slide contains a different diamine, wherein all spots on a given slide have the same diamine.

Step 4: Reaction of Mixed (2°-Amide-1°-amide-phenyl) Amine Intermediates with Carboxylic Acids.

The thirty slides from step 3 above were each treated with a solution of 3-iodo-4-methylbenzoic acid (0.25 M), 1-hydroxy-7-azabenztriazole (0.25 M), and diisopropylethylamine (0.5 M) in DMF. The reaction mixtures were agitated overnight before washing with DMF and methylene chloride to remove excess reagent.

At each stage in the coating assembly, XPS and ATR spectra were acquired and indicated that each transformation had been performed. Further, the assemble library of mixed (amide-amide-phenyl) system on a microarray was screened against anti Rabbit IgG, and a spectrum of very low to very high protein bindings events were observed.

6. *Synthon Coating: Carboxylated Polymer Bead Examples*

Synthon Coating Polymer

Inhibitor free styrene (86.4 mmol), maleic anhydride (86.4 mmol), and initiator AIBN (0.1mmol) were mixed together in 1,4-Dioxane (48ml) in a polymerisation ampoule and sealed with a rubber septum. The solution was degassed by nitrogen sparging then allowed to polymerise at 60°C in a temperature controlled oil bath. After an appropriate time interval the polymerisation was stopped by precipitation into a 10-fold excess of methanol. The copolymer was collected by filtration and purified once by reprecipitation into methanol from DMF. The alternating copolymer was characterised my GPC: Mw=270 000.

0.5 grams of the afforded polymer was dispersed into 50ml of Millipore water and hdrolyzed at 80°C with shaking over 5 days to afford the Synthon Coating Polymer, that is employed in the bead and plate examples below.

A) Absorption of the Synthon Coating Polymer

Step 1: A 100uL bead suspension of 5 micron, carboxylated was washed once with 2mls of Millipore water. The suspension was spun down and the bead plug resuspended into 1ml of a 1 wt% solution of PEI (Aldrich, 750K). The PEI was allowed to adsorb for 30 minutes with occasional gentle shaking and subsequently washed vigorously 3 times with Millipore water and spun down to a bead plug. The PEI coated beads were then resuspended in 1ml of 1% hydrolysed Synthon Coating Polymer 1 (described above) and allowed to adsorb for 30min with occasional gentle shaking. The beads were then washed 3 times with Millipore water with each washing step including 20min of gentle shaking and spun down to a bead plug.

Step 2: To effect the next coating stage, the spun down bead plugs with the PEI and adsorbed Synthon Coating Polymer were resuspended into 1ml of a 5mg/ml EDC water solution and after 1min, 25uL of the 1,5 pentyl diamine was added. The samples were

shaken briefly and the coupling reaction was allowed to proceed for 2 hrs with occasional gentle shaking. As the beads tended to clump during this process, they were redispersed with a short stints in the ultrasonic bath. The diamine coupled beads were then washed exhaustively with Millipore water 5 times and spun down to a bead plug. These amine modified beads were resuspended into 1ml of water and 200uL of the, 3-iodo-4-methyl-benzoic acid, sulfo-NHS ester (~10mg/ml of DMF) was added. The reaction was left to proceed for 2hrs and were then exhaustively washed 5 times with Millipore water. It should be noted that this modification can be effected by any number of diamines (or other multi-amine building block) and carboxylic acids, to allow the generation of libraries of modified encoded beads from the single Synthon Coating Polymer modified bead.

At each stage in the coating assembly, XPS spectra were acquired and indicated that each transformation had been performed. This process was performed on a number of beads sets from Bangs Laboratories (L020621N ,L020325G& Dyed: L011009A) and Luminex (L100-C124-01)

B) Covalent Attachment of the Synthon Coating Polymer

Step 1: A 100uL bead suspension of 5 micron, carboxylated was washed once with 2mls of Millipore water. The suspension was spun down and the bead plug resuspended into 1ml of a 1 wt% solution of PEI (Aldrich, 750K). The PEI was allowed to adsorb for 30 minutes with occasional gentle shaking and subsequently washed vigorously 3 times with Millipore water and spun down to a bead plug. The covalent attachment of the Synthon Coating Polymer to the PEI coated beads was performed by resuspending the PEI beads in 1ml of 1% Synthon Coating Polymer (preparation described above) that had been activated with EDC, and the reaction allowed to proceed for 30min with occasional gentle shaking. The beads were then washed 3 times with Millipore water with each washing step including 20min of gentle shaking and spun down to a bead plug.

Step 2: To effect the next coating stage, the spun down bead plugs with the PEI and adsorbed Synthon Coating Polymer were resuspended into 1ml of a 5mg/ml EDC water

solution and after 1min, 25uL of the 1,5 pentyl diamine was added. The samples were shaken briefly and the coupling reaction was allowed to proceed for 2 hrs with occasional gentle shaking. As the beads tended to clump during this process, they were redispersed with a short stints in the ultrasonic bath. The diamine coupled beads were then washed exhaustively with Millipore water 5 times and spun down to a bead plug. These amine modified beads were resuspended into 1ml of water and 200uL of the, 3-iodo-4-methyl-benzoic acid, sulfo-NHS ester (~10mg/ml of DMF) was added. The reaction was left to proceed for 2hrs and were then exhaustively washed 5 times with Millipore water. It should be noted that this modification can be effected by any number of diamines (or other multi-amine building block) and carboxylic acids, to allow the generation of libraries of modified encoded beads from the single Synthon Coating Polymer modified bead.

At each stage in the coating assembly, XPS spectra were acquired and indicated that each transformation had been performed. This process was performed on a number of beads sets from Bangs Laboratories (L020621N ,L020325G& Dyed: L011009A) and Luminex (L100-C124-01)

C) Multiplex Bead Based Assay

Encoded Carboxylated beads employed in the assay were acquired from Luminex, and treated with Step 1 of the Absorption of the Synthon Coating Polymer described above. 5.0 X 10^6 microspheres were transferred to a 15mL microcentrifuge tube, spun down to a pellet and resuspended in 5mL of 0.1M MES, pH 4.5 making sure to vortex and sonicate beads well.

0.2 nmol of capture oligo probes (2mL of 1:10 of stock in dH20) was added to the beads, followed by a fresh aliquot of 10mg/mL EDC in dH20 (2.5mL). The reaction was allowed to proceed for 30 minutes at room temperature in the dark, prior to washing and charging the vessel with another fresh solution of 2.5mL of EDC. This solution was also incubated for 30 minutes at room temperature in the dark, then washed with 1.0mL of 0.02% Tween-20. The suspension was centrifuged for 1 minute to produce pellet and the supernatant

carefully removed. The beads were then washed with 1.0mL of 0.1% SDS, centrifuged for 1 minute to produce pellet and the supernatant carefully removed. The beads were then finally suspended in 100mL of TE, at pH 8.0 and stored at 2-8°C in complete darkness.

The coupled beads were then resuspended 1.5 X TMAC buffer and distributed to a sample or background well on the PCR plate. The amplified biotinylated DNA was then added and TE, pH 8.0 added to make a total of 17mL. The solutions were gently pipet up and down to mix. The samples were covered with plate sealer and place in thermocycler under a program that is set at 95°C (denaturing step) for 5 minutes and then 52°C (hybridization step) for 15 minutes. The plate was then spun (³2250 x g, 3 minutes) and the supernatant carefully removed, and the plate placed back into the PCR at 52°C. 75mL of reporter solution was then added to each well, mixed gently and incubate at 52°C for 5 minutes prior to analysis via a Luminex machine, to afford an improved signal to noise over the non-modified Encoded Carboxylated beads.

*7. **Coating of a Multi-well Plate***

A) Non-Reactive Microtitre Plate

Step 1: 200uL of a 1wt% PEI (Aldrich, 750K) was added to the wells of a 96 well microtitre plate (Maxisorp, Nunc) and allowed to stand at room temperature for 60 min. The wells were then washed 5 times with Millipore water. 200uL of a 1wt% Synthon Coating Polymer (preparation described above) was added to the wells and the interaction allowed to proceed for 60 min. The wells were then washed 5 times with Millipore water.

Step 2: 200uL of a 5vol% 1,5 pentyl diamine in 5mg/ml EDC water solution was added to the wells and coupling allowed to proceed for 2hrs, and then the wells were washed 5 times with Millipore water. 200uL of a coupling solution comprising 5mg/ml EDC and 5mg/ml 3-iodo-4-methyl-benzoic acid in DMSO was added to the wells and allowed to proceed for 2 hours after which the wells were washed twice with fresh DMSO then 5 times with Millipore water.

It should be noted that this modification can be effected by any number of diamines (or other multi-amine building block) and carboxylic acids, to allow the generation of libraries of modified microtitre plate wells from a single Synthon Coating Polymer modified bead.

At each stage in the coating assembly, XPS spectra were acquired and indicated that each transformation had been performed. The modified plates could then be employed in standard immunoassay protocols for ELISA and other diagnostic procedures

B) Reactive Microtitre Plate

Step 1: 200uL of a 1wt% Synthon Coating Polymer (preparation described above) was added to the wells NHS active plate, DNA-BIND (Corning) and ReactiBind plate (Piece) and the reaction allowed to proceed for 60 min. The wells were then washed 5 times with Millipore water.

Step 2: 200uL of a 5vol% 1,5 pentyl diamine in 5mg/ml EDC water solution was added to the wells and coupling allowed to proceed for 2hrs, and then the wells were washed 5 times with Millipore water. 200uL of a coupling solution comprising 5mg/ml EDC and 5mg/ml 3-iodo-4-methyl-benzoic acid in DMSO was added to the wells and allowed to proceed for 2 hours after which the wells were washed twice with fresh DMSO then 5 times with Millipore water.

It should be noted that this modification can be effected by any number of diamines (or other multi-amine building block) and carboxylic acids, to allow the generation of libraries of modified microtitre plate wells from a single Synthon Coating Polymer modified bead.

At each stage in the coating assembly, XPS spectra were acquired and indicated that each transformation had been performed. The modified plates could then be employed in standard immunoassay protocols for ELISA and other diagnostic procedures.

8. Coating of PVDF Membrane

Step 1: Activation of the Membrane with a Grafted Synthon Polymer

Four, 10 x 20 cm pieces of Immobilon-P^{SQ} PVDF membrane (Millipore) were placed into a 700 ml beaker. The beaker was filled with a 1.5M ethyl acetate solution of 1:1 Styrene and Maleic anhydride, degassed by nitrogen purging and sealed. The solution was then irradiated in a gamma cell for 100 min. The irradiated membranes were removed from the polymerisation solution and washed with a large excess of ethyl acetate. Once washing was complete, the membranes were dried under high vacuum overnight and stored in a low humidity cupboard.

Step 2: Modification of the Membrane

A standard solution of the amine in THF (100 ml, 0.25 M, 0.025 mol) was prepared for each amine used. Grafted PVDF membranes were cut to a size of 10 x 10 cm, and placed in a large Petrie dish. The 100 ml amine solution was then carefully poured into the Petri dish, ensuring that the membrane was fully wet. The Petri dishes were then sealed with lids and allowed to agitate (very slowly) overnight at room temperature. The reaction solution was removed from the petri dish and the membranes washed with THF, dried under vacuum overnight and stored in the low humidity cupboard.

It should be noted that this modification can be effected by any number of amines (or other multi-amine building block) to allow the generation of libraries of modified PVDF membranes from a single grafted Synthon Polymer modified membrane.

At each stage in the coating assembly, XPS and ATR spectra were acquired and indicated that each transformation had been performed. The modified plates could then be employed in standard electroblotting protocols for western blotting applications to increase the amount of captured protein available for immunoassay.

9. Synthon Coating: Determining the Optimium Coating for a Desired Specific Application

Step 1: Preparation of Library on Desired Format:

A library of different but related surfaces are assembled in the desired format (microarray, bead, plated, etc) for the application, employing the methods described above.

Step 2: Screening of the Assembled Library

The assembled libraries are screened against the desired target for the desired application such as a biological screen for kinases, Rabbit IgG, cytokines or a synthetic screen for reaction optimizations, or the like. The outcome from this screen would be to identify the optimum surface for the said desired application, in a rapid and cost effective manner.

If the desired level of signal is not attained from the first screen of the libraries, a second, more focused library is then assembled with the knowledge from the first and the screen repeated until the desired level of signal is obtained. More than one surface from each screen may afford a signal of the desired level.

Step 3: Generation of a Synthon coating for a Desired Specific Application.

Having determined the optimum surface for the desired application, the identified surface can then be assembled by any means required, that affords the surface in a timely and cost effective manner. Further, the outcomes of a number of screening events can be assembled onto one surface, such as a microarray, resulting in a multiplex platform having, or consisting of multiple elements or parts to do more than one experiment.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications which fall within its spirit and scope. The invention also includes all the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

CLAIMS

1. A method of generating a library of different surface coatings on a substrate comprising:

a) selecting a surface coating synthon of formula B-S-F, wherein B is a copolymer of at least one passive constituent P and at least one active constituent A, S is a spacer unit and F is a chemical or biological functional group, wherein S is attached to an active constituent A of copolymer B, and wherein the synthon has at least one point of diversity selected from P, A, S and F;

b) applying backbone coating(s) of the selected copolymer B onto a substrate;

c) attaching the selected combination(s) of spacer unit S and functional group F to an active constituent A of copolymer B according to said selected synthon;

wherein steps b) and c) are performed such that surface coatings according to the synthon are generated on localised regions of the substrate, thereby providing said library of different surface coatings on the substrate.

2. The method according to claim 1, wherein the substrate is selected from an organic polymer, glass, silicon, metal and combinations thereof.

3. The method according to claim 1, wherein the substrate is in the form of a microscope slide, microtitre plate, porous membrane, pipette tip, tube or a plurality of beads.

4. The method according to claim 2, wherein the substrate is an organic polymer.

5. The method according to claim 4, wherein the organic polymer is selected from

polytetrafluoroethylene, polystyrene, polypropylene, polyethylene, polyvinylidenefluoride and polymethylmethacrylate.

6. The method according to claim 4, wherein the organic polymer is in the form of a plurality of beads.

7. The method according to claim 6, wherein the beads are labelled such that a particular coating can be related to a particular bead or subgroup of beads.

8. The method according to claim 7, wherein the beads are Luminex™ beads.

9. The method according to any one of claims 1 to 8, wherein the library comprises at least 10 different surface coatings.

10. The method according to any one of claims 1 to 8, wherein the library comprises at least 100 different surface coatings.

11. The method according to any one of claims 1 to 8, wherein the library comprises at least 1,000 different surface coatings.

12. The method according to any one of claims 1 to 8, wherein the library comprises at least 10,000 different surface coatings.

13. The method according to any one of claims 1 to 12, wherein the active constituent A of copolymer B is a polymerised residue of a compound selected from those listed in Table 1 of this specification.

14. The method according to claim 13, wherein the active constituent A of copolymer B is a polymerised residue of maleic anhydride.

15. The method according to any one of claims 1 to 14, wherein the passive constituent

P of copolymer B is a polymerised residue of a compound selected from those listed in Table 2 of this specification.

16. The method according to claim 15, wherein the passive constituent P of copolymer B is a polymerised residue of styrene.

17. The method according to any one of claims 1 to 16, wherein copolymer B is an alternating copolymer.

18. The method according to any one of claims 1 to 17, wherein copolymer B is a block copolymer of the active constituent A and the passive constituent P.

19. The method according to any one of claims 1 to 18, wherein copolymer B is a copolymer of maleic anhydride and styrene.

20. The method according to any one of claims 1 to 19, wherein copolymer B further comprises a control agent C.

21. The method according to claim 20, wherein the control agent is selected from a RAFT control agent, an ARTP control agent and a nitroxide control agent.

22. The method according to any one of claims 1 to 21, wherein the spacer unit S has the structure:

$$X - Q - Y$$

wherein X is the residue of an amino, hydroxyl, thiol, carboxylic acid, anhydride, isocyanate, sulfonyl chloride, sulfonic anhydride, chloroformate, ketone or aldehyde moiety; Y is the same as defined for X; and Q is a divalent organic group, and wherein X and Y are not reactive with each other or Q.

23. The method according to claim 22, wherein Q is selected from optionally substituted C_1 to C_{20} alkylene, optionally substituted C_2 to C_{20} alkenylene, optionally substituted C_2 to C_{20} alkynylene and optionally substituted C_6 to C_{20} arylene, wherein one or more carbon atoms may be substituted with a heteroatom selected from O, S or N.

24. The method according to claim 22 or claim 23, wherein the spacer unit S is a residue of a diamine.

25. The method according to claim 24, wherein the spacer unit S is a residue of an alkyl diamine.

26. The method according to claim 25, wherein the spacer unit S is a residue of 1,5-diaminopentane or N-(3-aminopropyl)-1,3-propanediamine.

27. The method according to any one of claims 1 to 26, wherein the chemical or biological group F is a group capable of binding or chemically reacting with a biological molecule or component.

28. The method according to claim 27, wherein the chemical or biological group F comprises a primary or secondary amine group.

29. The method according to any one of claims 1 to 28, wherein the synthon has, within the active constituent A, the passive constituent P, the spacer unit S and the functional group F, a sole point of diversity in the selection of the spacer unit S.

30. The method according to any one of claims 1 to 28, wherein the synthon has, within the active constituent A, the passive constituent P, the spacer unit S and the functional group F, a sole point of diversity in the selection of the functional group F.

31. The method according to any one of claims 1 to 28, wherein the synthon has, within the active constituent A, the passive constituent P, the spacer unit S and the

functional group F, two points of diversity in the selection of the spacer unit S and the functional group F.

32. The method according to any one of claims 1 to 31, wherein the backbone coating(s) of copolymer B are applied onto the substrate by grafting, or other methods of coating selected from dip coating, plasma polymerisation, vapor deposition, stamp printing, gamma irradiation, electron beam exposure, and thermal and photochemical radiation.

33. The method according to any one of claims 1 to 32, wherein the selected combination(s) of spacer unit S and functional group F are attached by:

1) attaching the spacer unit S to copolymer B and then attaching the functional group F to the attached spacer group S; or
2) attaching the spacer unit S to copolymer B, wherein the spacer unit S already has the functional group F attached to it.

34. The method according to any one of claims 1 to 33, wherein the backbone coating(s) of selected copolymer B is applied onto localised regions of the substrate.

35. The method according to claim 34, wherein the backbone coating(s) of selected copolymer B is applied to a plurality of beads.

36. The method according to any one of claims 1 to 33, wherein the backbone coating(s) of selected copolymer B is applied to the surface of the substrate, and the selected combination(s) of spacer unit S and functional group F are attached to the copolymer B in localised regions.

37. The method according to any one of claims 1 to 36, wherein the surface coatings according to the synthon which are generated on localised regions of the substrate are spatially resolved.

38. A method of optimizing a substrate surface for a solid-phase application involving immobilization of a molecule comprising:

a) generating a library of different surface coatings on a substrate by a method comprising:

1) selecting a surface coating synthon of formula B-S-F, wherein B is a copolymer of at least one passive constituent P and at least one active constituent A, S is a spacer unit and F is a chemical or biological functional group, wherein S is attached to an active constituent A of copolymer B, and wherein the synthon has at least one point of diversity selected from P, A, S and F;

2) applying backbone coating(s) of the selected copolymer B onto a substrate;

3) attaching the selected combination(s) of spacer unit S and functional group F to an active constituent A of copolymer B according to said selected synthon;

wherein steps 2) and 3) are performed such that surface coatings according to the synthon are generated on localised regions of the substrate, thereby providing said library of different surface coatings on the substrate;

b) exposing at least two of the surface coatings in the library to the molecule to be immobilized; and

c) determining which of the at least two surfaces results in better performance of the immobilized molecule in the solid-phase application.

39. The method of claim 38 wherein the solid-phase application involves immobilization of a biological molecule or a biological molecule analog selected from proteins, peptides, peptide nucleic acids, nucleic acids, non-natural nucleic acids, oligonucleotides and carbohydrates.

40. The method according to claim 38, wherein the solid-phase application involves detecting binding of a ligand to an immobilised biological molecule.

41. A solid phase application involving immobilisation of a biological molecule, wherein the biological molecule is immobilised on a substrate surface optimized by the method of claim 38.

42. A biological molecule detection unit capable of detecting at least two biological molecules, said unit comprising a substrate having a plurality of surface coatings wherein at least two of said coatings are different, and tailored to recognise, bind to or associate with a particular biological molecule.

AMENDED CLAIMS

[Received by the International Bureau on 12 September 2003 (12.09.03);
New claim 43 added, remaining claims unchanged (1 page)]

41. A solid phase application involving immobilisation of a biological molecule, wherein the biological molecule is immobilised on a substrate surface optimized by the method of claim 38.

42. A biological molecule detection unit capable of detecting at least two biological molecules, said unit comprising a substrate having a plurality of surface coatings wherein at least two of said coatings are different, and tailored to recognise, bind to or associate with a particular biological molecule.

43. A method of tailoring a surface coating to recognise, bind to or associate with a particular biological molecule comprising:

a) generating a library of different surface coatings on a substrate by a method comprising:

1) selecting a surface coating synthon of formula B-S-F, wherein B is a copolymer of at least one passive constituent P and at least one active constituent A, S is a spacer unit and F is a chemical or biological functional group, wherein S is attached to an active constituent A of copolymer B, and wherein the synthon has at least one point of diversity selected from P, A, S and F;

2) applying backbone coating(s) of the selected copolymer B onto a substrate;

3) attaching the selected combination(s) of spacer unit S and functional group F to an active constituent A of copolymer B according to said selected synthon;

wherein steps 2) and 3) are performed such that surface coatings according to the synthon are generated on localised regions of the substrate, thereby providing said library of different surface coatings on the substrate;

b) exposing at least two of the surface coatings in the library to the particular biological molecule; and

c) determining which of the at least two surfaces best recognises, binds to or associates with the particular biological molecule.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU03/00566

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C07K 17/02, 17/06, C09D 135/06, G01N 33/547

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CA, WPIDS, MEDLINE; KEYWORDS: COPOLYMER, ORTHOGONAL, COMBINATIONAL, ARRAY, LINKER, LIBRARY

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	US 6403368 B1 (Jan et al) 11 June 2002 Figures 1, 10, column 5 line 59 - column 6 line 18, claims	1-5, 9-42
P,X P,Y	US 6515039 B1 (Ulbricht et al) 4 February 2003 (& WO 00/12575 A1). Column 1 line 50- column 2 line 32, Figure 1, column 6 line 25 - column 7 line 29, column 8 line 20- column 9 line 26, examples, claims.	1-5, 9-13, 15-18, 20-23, 27-28, 30, 32-34, 36-42 6-8

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 June 2003	01 JUL 2003
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **ROSS OSBORNE** Telephone No : (02) 6283 2404

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU03/00566

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 02/50171 A (POLYMERAT PTY LTD) 27 June 2002 Page 15 line 10 - page 24 line 4, claims 44-50	1,2, 4-5, 27-28, 32, 38, 42
X Y	US 6346413 B1 (Fodor et al) 12 February 2002 See figures, in particular figure 3, column 2 line 51-column 6 line 42 Column 6 lines 25-33	42 6
X Y	US 5922545 A (Mattheakis et al) 13 July 1999 Claims Claim 2	42 6-8
X	US 6329209 B1 (Wagner et al) 11 December 2001 Figures 1-7, column 3 lines 10-16, column 16 lines 8-28, claims	42
A	US 2002/0025380 A (Vanmaele et al) 28 February 2002 Whole document	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU03/00566

Box I **Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos :

because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. Claims 1-41 directed at methods of generating libraries of surface coatings based on a synthon B-S-F.

2. Claim 42 directed at a biological molecule detection unit having a plurality of surface coatings.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU03/00566

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	6403368	US	2002122875	US	2002106785	US	2002182719
US	6515039	EP	1027379	WO	2000/12575	AU	58556/99
WO	2002/50171	AU	2002/15694				
US	6346413	AU	58371/90	AU	77655/94	BR	9007425
		CA	2054706	EP	476014	EP	619321
		EP	902034	EP	953835	HK	613/95
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		US	6124102	US	6225625	US	6309822
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END OF ANNEX